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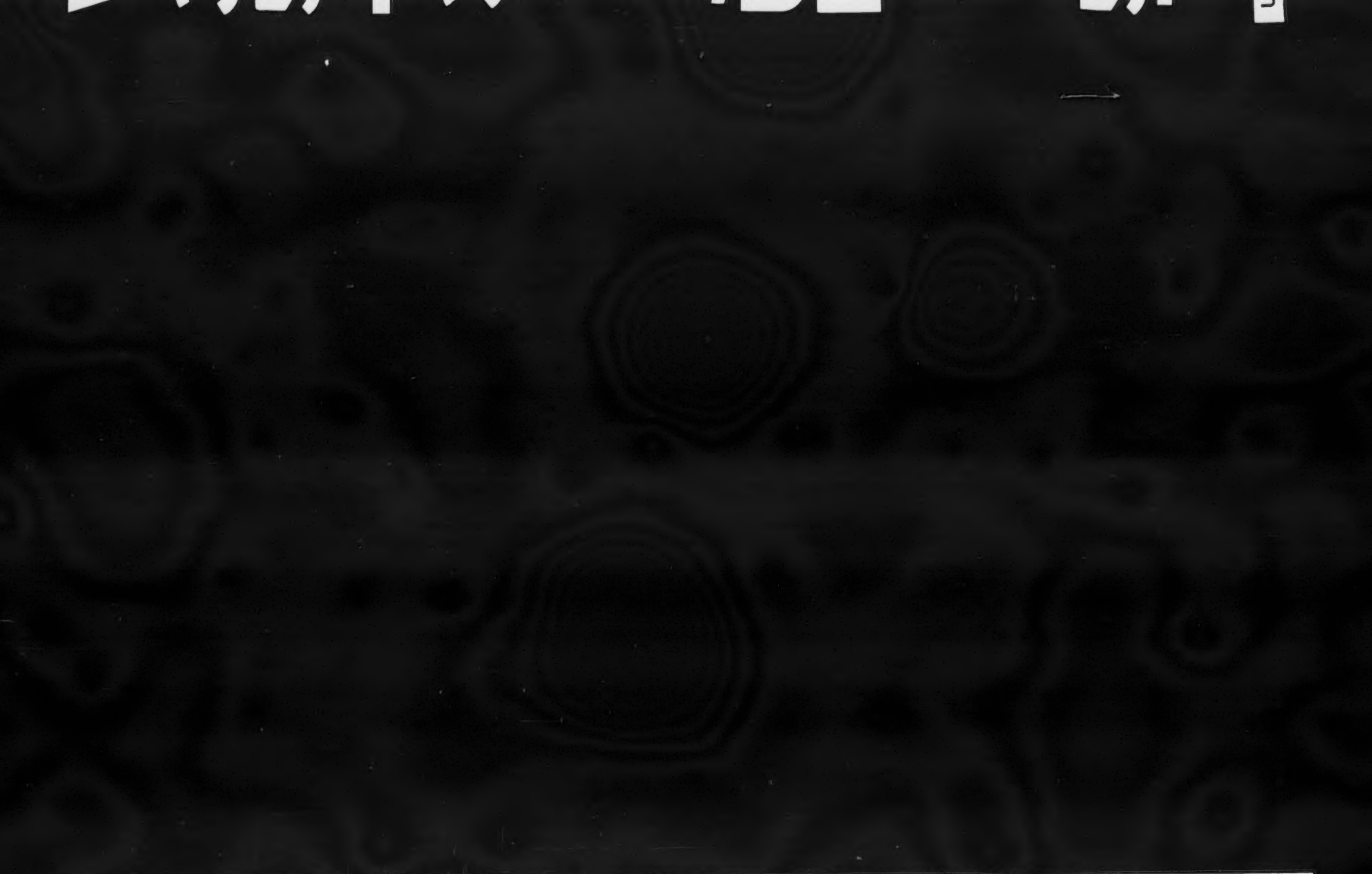

CORRECTIONS

Can. J. Zool. 35, 75–92 (1957). In Figs. 1 and 2 the oesophageal gland lobe should overlap the intestine ventrally and the caudal alae in Fig. 2 should point towards the bottom of the page. The error has been caused by the wrong juxtaposition of three components of the original drawing, which, being too large to be drawn in one field, had to be constructed in several small components. In Fig. 8 there should be only four incisures as described in the text.

Can. J. Zool. 34, 583–594 (1956). In line 13, page 590, "A cirrus was not seen in 17 of 26 flukes examined." should read "A cirrus was seen in 17 of 26 flukes examined."

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INDIVIDUAL DIFFERENCES AS A FACTOR IN POPULATION DYNAMICS: THE DEVELOPMENT OF A PROBLEM[1]

W. G. WELLINGTON[2]

Abstract

The role of individual differences in the population dynamics of animals has been relatively neglected by ecologists. Individual requirements and responses to environmental pressures differ, however, and it is unwise to assume that the range of this variation within a population remains constant through successive generations. Moreover, the assumption that successive changes in the range of variation might affect the subsequent efficiency of some factors believed to regulate population density is worth further consideration. It cannot be tested adequately, however, by considering only the amounts of mortality that different extrinsic factors cause within a generation. Its adequate assessment also requires detailed observations of the qualities of different types of individuals. Therefore, in order to assess the importance of individual variation in the population dynamics of an insect, an outbreak of the western tent caterpillar, *Malacosoma pluviale* (Dyar), was studied in detail during 1956. Larvae were classified by differences in their total activity and behavior on emergence, and the proportions of the different types per egg mass were determined. Differences obtained were found to be associated with different feeding habits and rates of food consumption and development as well as with different chances for survival in particular environments or in the presence of disease. Colonies in new infestations consisted largely of active individuals, whereas less active individuals occurred with increasing frequency as infestations became older and heavier. Adult activity also varied, and more active moths appeared to be responsible for production of active colonies, especially in new infestations most distant from original sources. The population must be followed through its eventual decline to its next increase before complete information can be obtained, but present evidence is sufficient to formulate an acceptable working hypothesis, namely, that proportions of the different types within the population will show annual as well as areal changes. Moreover, the fact that the insect is colonial is not especially significant, since similar differences in total activity also may be detected among other species with solitary habits.

Introduction

Populations are composed of individuals, and individuals differ. Nevertheless, our attempts to identify and evaluate factors regulating animal numbers frequently seem to include a tacit assumption that the populations thus regulated are monolithic, or else consist of well-nigh interchangeable units that respond uniformly to given biotic or physical pressures. Thus, students of

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population dynamics seldom determine the proportions of physiologically or genetically different groups within a generation or allow for the possibility that these proportions might change between generations as the population density changes.

The idea that the composition of a population might change with changing density is not new (Chitty (1)), but population ecologists have remained remarkably resistant to its theoretical and practical implications. Nevertheless, recent evidence (1) and arguments (2, 7) for some of its aspects suggest that it merits closer attention. Moreover, since our existing hypotheses continue to be more persuasive in print than in the field, analyses of intraspecific variation might help to improve our evaluations of the roles of both physical and biotic factors in population dynamics. In short, our theory should be based on detailed analyses of the animals as well as the environmental factors that affect them.

Many kinds of insects provide excellent material for such analyses. For example, tent caterpillars (*Malacosoma* spp.) fluctuate violenly in numbers in many parts of North America, reaching peaks at roughly 8-year intervals. Their changes in abundance have been ascribed to various agents, including parasites, disease, climate, and solar phenomena. None of these provides an adequate explanation by itself, but selection of more comprehensive hypotheses is still largely a matter of taste. In 1956, a continuing outbreak of the western tent caterpillar, *M. pluviale* (Dyar), in southern Vancouver Island, British Columbia, provided unusual opportunities for determining variability within populations as well as for evaluating the importance of parasites, diseases, food, and climate as regulating factors. Results will be reported from time to time, but the present paper summarizes those from 1956 that demonstrate ways in which individual differences may be important in population dynamics. Since topics range from sensory physiology to population density, many details of techniques and results have been compressed or omitted to provide space for points of more general interest.

Life History

Like other tent caterpillars, individuals of *M. pluviale* overwinter as fully developed embryos in egg masses attached to twigs of their food trees, notably *Alnus*, *Salix*, and orchard trees. Numbers of first-instar larvae vary from less than 100 to nearly 300 per egg mass, and emergence dates in one locality also are highly variable, ranging from early April to late May in response to seasonal weather. On emergence, the larvae are colonial, congregating on the surface of the egg mass for a day or more, or moving in groups along the twigs to leaves. They lay silk trails as they travel, and soon begin to construct a communal tent that serves as a resting and molting shelter from which they emerge to feed. Their clustering and processionary tendencies are retained through most of their larval life, weakening only during the last instar, when individuals begin to leave a colony prior to pupation. Pupation occurs in

June or early July, and takes place in cocoons spun in rolled leaves, on twigs, debris, or buildings after extensive prepupational travel by the mature larvae. The winged adults emerge in July or, more rarely, in early August.

Differences on Eclosion

In the field, group behavior is so striking that observers tend to disregard the actions of individuals. In the laboratory, however, individual differences soon become apparent when the behavior of single larvae is observed. For example, if larvae emerging from an egg mass are placed separately on a flat surface so that they cannot contact one another, only a few seem capable of directed movement toward a light source; the majority either circle aimlessly or remain stationary for long periods. This difference was first encountered during a comparative study of the light reactions of *M. disstria* Hbn., *M. americanum* (Fab.), and *M. pluviale*, where it was marked enough in first-instar larvae of all three species to require modification of standard tests (12). At the time, it was not possible to determine whether individuals maintained their differences or simply alternated between sluggish and active periods. Nevertheless, the known difference provided a convenient point from which to develop the present investigation.

Thirty egg masses collected in August, 1955, provided material for laboratory tests. They were stored at 4° C. and 78% R.H. during the winter, and yielded 4581 larvae in the spring of 1956. Initial separation of individual larvae from one egg mass on the basis of their ability to perform directed movements in response to light was accomplished most rapidly by placing the larvae on white paper in rows of 30 parallel to a 30 watt fluorescent tube. Individuals were spaced so that they could not contact one another, and observed for 10 minutes. Those that moved directly toward the light were separated from the others. Finally, the remaining larvae were brushed into clusters and their further actions were observed for another 10 minutes.

Hereafter, individuals capable of independent, directed movements are termed Type I larvae. Type II includes several categories. The first of these includes a few larvae that were incapable of independent directed movement because the amplitude of their body swings in the absence of a silk trail was so great that they turned too frequently to proceed very far in one direction. On a silk path, or alongside another larva blocking lateral movements of their bodies, they travelled a straighter course. The second, major group of Type II larvae swayed so much and so frequently in the absence of paths that they turned aimlessly. Alongside larvae of the same type they accomplished little more, but they followed directed larvae or the trails that these left very precisely and rapidly. The last category of Type II consists of sluggish larvae that seldom did more than move their heads while isolated and, indeed, moved less often than the other types even when they were in contact with them.

Repeated tests over a 3 day period showed that these differences were persistent. Moreover, in the first instar they were stable from 15°–32° C., the temperature range in which directed movement is apt to occur in fed and

starved individuals. Increases in light intensity to full sky values increased the radius of turning of active, undirected larvae but did not eliminate turning as such. The results of these major differences in behavior when larvae are grouped are best illustrated by the series of photographs, Figs. 1–26.*

Figs. 1–4 show the actions of 14 Type I larvae 24 hours old when they were placed on clean white paper in a beam of light. The photographs show the directed movements in a gradient of light intensity, and the tendency to react independently whether or not silk trails are present at the beginning of movement.

Figs 5–8 show the contrasting behavior of 16 active Type II larvae of similar age. In the absence of paths, pure groups of Type II larvae characteristically form closely packed clusters, provided they are spaced closely enough to contact one another when they begin to make lateral movements with their bodies.

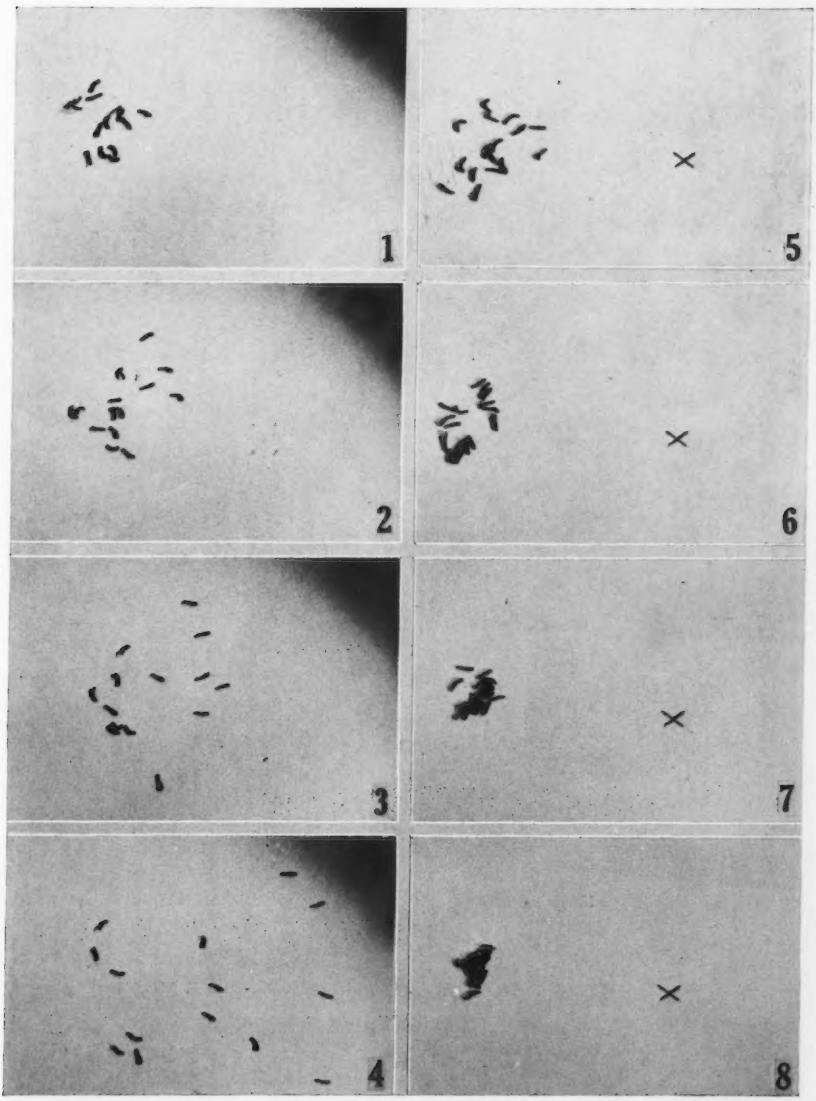
After the photograph for Fig. 8 was obtained, a single Type I larva was placed near the upper boundary of the cluster. The subsequent photographs show: (*a*) the members of the cluster beginning to follow the Type I larva, its silk trail, or the silk deposited over this trail by the first few larvae in the line (Figs. 9–12); (*b*) the initial effects of *removal* of the Type I larva prior to Fig. 13, and the slow return of the original group from the finish of the Type I trail to the vicinity of the original clustering site (Figs. 14–19); (*c*) continued activity of the Type II group resulting in partial return to the end of the trail, but not beyond it (Figs. 20–23), and (*d*) formation of two final clusters near the two ends of the silk path (Figs. 24–26).

Thus, in the absence of a silk trail, Type II larvae tend to aggregate whenever they are close enough to make contact, just as they tend to become quiet when they are isolated. Nevertheless, active Type II larvae will use a trail if it is already available, though in the absence of additional stimuli they tend to spend more time clustering than travelling.

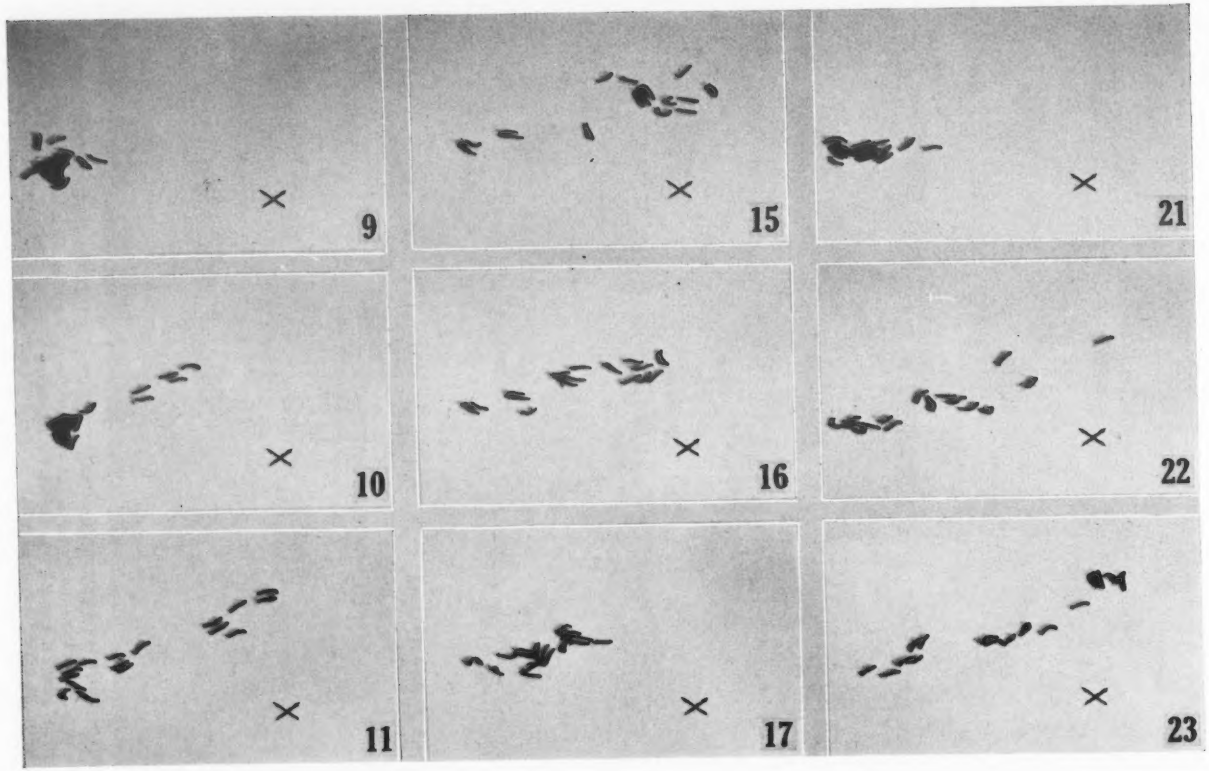
Although it was possible to establish the fact that different egg masses contained different proportions of the various types of individuals, it was impossible to demonstrate orderly trends in the data obtained during 1956. The egg masses had to be collected before there was any basis for judging the suitability of collecting areas, and mixed collections produced inconsistencies. The proportions of Type I larvae per egg mass varied from 0–38%, but analyses attempting to relate these differences to the number of eggs per mass, the percentage emergence, and the percentages of sterile, incompletely developed, or parasitized eggs all indicated that collections had been made in

*Unless specifically stated, each experimental group photographed in the laboratory or the field was made up within a colony; i.e., the differences illustrated occurred between members of a colony as well as between those from different egg masses.

FIGS. 9–26. The behavior of the Type II first-instar larvae from FIGS. 5–8 after one Type I larva was first added to the cluster and later removed. FIGS. 9–12 at 45-second intervals after the introduction of the Type I larva; FIG. 13, 20 seconds after the *removal* of the Type I larva; FIGS. 14–19, 2, 3, 6, 8, 9, and 10 minutes after the Type I larva was removed; FIG. 20, minute 12, and FIGS. 21–26 at 1-minute intervals thereafter.



FIGS. 1–8. Contrasting behavior of Type I (FIGS. 1–4) and active Type II (FIGS. 5–8) first-instar larvae of *M. pluviale* placed on clean white paper in a beam of light. Larvae were 24 hours old and averaged 2.8 mm. in length. FIGS. 1–4 at 0, 5, 10, and 20 seconds; FIGS. 5–8 at 0, 2, 9, and 10 minutes.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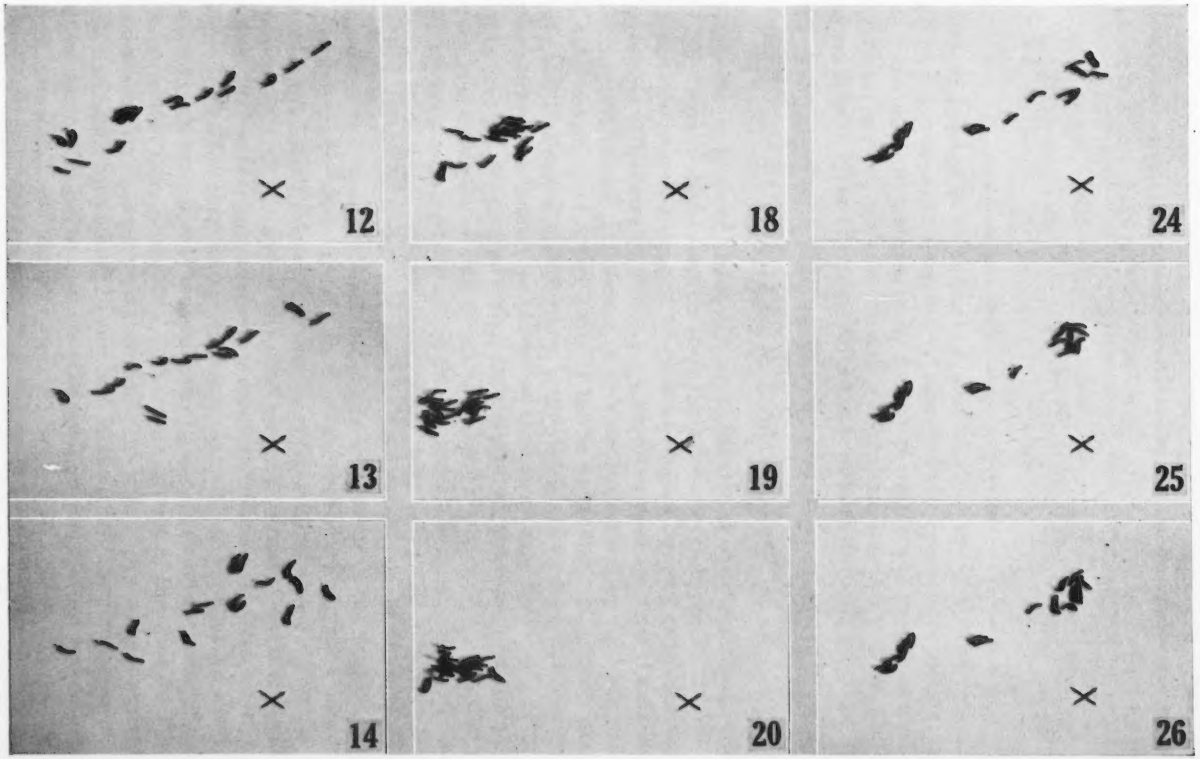

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TABLE I

EMERGENCE OF TYPE I AND TYPE II LARVAE FROM 30 EGG MASSES IN 1956

No./egg mass	Total eggs	Total hatch	No. Type I	No. Type II
$\bar{x}$	213.47	145.47	27.57	117.53
s	37.85	64.85	19.13	52.07
$s_{\bar{x}}$	6.91	11.84	3.49	9.51
Range	120–287	4–242	0–78	4–192

an area of overlap between two populations that could not be separated properly for analytical purposes. Consequently, apart from noting that Type I larvae showed no tendency to emerge during any particular part of the eclosion period, and that larger emergences frequently gave greater numbers of both types of larvae instead of invariably favoring one type, it is unwise to go beyond the records shown in Table I.

Differences in Establishment

The different types of first-instar larvae differed in their abilities to reach food and become established on it in the laboratory. From 20° to 30° C., both individuals and groups of 10 or more Type I larvae located leaves on twigs readily in darkness or in light. On the other hand, no Type II individual reached leaves only 8 cm. distant, a very short distance by field standards, and only one group of active Type II larvae succeeded. Nine other active groups and 10 sluggish groups never proceeded far from the deposition area, but spent more and more time clustering there until they died.

On twigs, the fate of mixed groups depended on their composition. Inclusion of even one Type I larva in a group of active Type II larvae permitted the group to reach a leaf over the trail established. When sluggish larvae were added to such a mixture, however, they were often left behind to die. Occasionally, active larvae left the leaf for brief returns to the deposition point, where they sometimes succeeded in stirring some of the sluggish larvae to greater activity that brought them to the leaves. Even then, however, some sluggish individuals were left to die at the original site.

In 6-cm. jars, all groups but the most sluggish located a single leaf within 24 hours. Type I larvae frequently reached it individually and seldom formed into groups during their initial feeding period. Consequently, the silk they deposited while moving over the leaf formed a very open network that afforded little protection from desiccation. Therefore, groups of less than 20 Type I larvae frequently became weakened and died unless they were combined in larger numbers. Groups of 20 survived if their daily accumulation of silk was not removed when the leaf was changed but was allowed to build up to provide protection from desiccation.

On the other hand, groups of active Type II larvae seldom reached a leaf individually, but made contact with it more or less accidentally by gradual extensions of the increasingly dense pad of silk they formed in the jar during

repeated expansions and contractions of their cluster. Once they established a bridge to the leaf, they began to feed in groups, so that the closely woven silk they produced together protected them from desiccation. Furthermore, they were much less apt to reject slightly wilted leaves than were Type I larvae, which commonly left such food and starved because they did so.

Sluggish groups in jars seldom reached a leaf, even when its edge was only 1 cm. distant. They remained immobile in clusters most of the time, with the result that they produced little silk. Therefore, those that did not starve died from desiccation. In fact, groups composed entirely of sluggish Type II larvae could be established and maintained only when they were deposited directly on a leaf. Even then, those that dropped off periodically had to be replaced or they would starve less than a centimeter from their food.

Retention of Differences in Responses

When first-instar larvae moved at all, direct observation of the amount and kind of movement was generally sufficient to distinguish the different types. No individual moved constantly, however, and was, in fact, subject to well-defined rest periods between feeding periods. Consequently, it was not always possible to tell immediately whether a larva was a sluggish Type II or a resting Type I. More directed larvae reacted quickly to disturbance, however, and began to exhibit their characteristic behavior after a minute or two of intermittent disturbance. Therefore, when larvae were not resting, some of the differences between Types I and II could be expressed quantitatively. During 1956, measurements were made at 21° C. and 60% R.H. while first-instar larvae were 30 cm. from a 30 watt fluorescent lamp.

Lateral movements of the fore part of the body 45° or more off the line of travel provided the most reliable distinction, since Type I and Type II larvae averaged 0.94 and 12.98 swings per minute, respectively. Consequently, any travelling larva that made three or more such swings per minute could be safely assigned to Type II without additional tests. Lateral movements of less than 45° and rates of crawling were less reliable, averaging 1.81 and 3.32 swings per minute and 23.32 and 22.14 mm. per minute, respectively. Furthermore, neither the number of lateral movements nor the rate of travel were always reliable indicators of differences between active and sluggish Type II larvae if the latter could be induced to move at all. Therefore, separation of these categories often entailed recurrent observations of their total activity and their ability to establish themselves on food without assistance during the first 24 hours after emergence.

As larvae pass through subsequent instars and increase in size, the capacity for independent, directed movement appears among both active and sluggish Type II individuals. This is associated with some reduction in the number of lateral comparisons per minute as well as with a greatly increased rate of travel. Nevertheless, the basic differences remain when larvae are moving in gradients of light intensity or on uniformly lit flat surfaces. Some of the more obvious distinctions are best shown by further series of photographs.

TABLE II

QUANTITATIVE COMPARISONS OF THE ACTIONS OF TYPE I AND TYPE II LARVAE ILLUSTRATED IN FIGS. 27–35

	Larval type		
	I	Active II	Sluggish II
Mm. from release point to lamp	581	575	556
Mm. travelled to lamp	596	667	1179
Time to reach lamp	1 min. 0 sec.	2 min. 20 sec.	3 min. 50 sec.
$\bar{x}$ rate of travel, mm./10 sec.	99.33±8.69	47.64±3.71	51.26±4.76
$\bar{x}$ no. of body movements <45°/10 sec.	0.67±0.36	1.27±0.20	0.83±0.21
$\bar{x}$ no. of body movements >45°/10 sec.	0.00	1.14±0.21	1.65±0.33
Time intervals (n)	6	14	23

Figs. 27–35 show the responses of three fifth-instar larvae to a 6 watt lamp in a dark room at 21° C. and 65% R.H. The Type I larva on the left travelled the 58 cm. distance in 1 minute, and made no comparative movements of 45° or more during the trip. The active Type II larva in the center required nearly 3 minutes to reach the lamp, and made 24 comparative movements of 45° or more. The sluggish Type II larva on the right did not reach the lamp in 4 minutes, but during that time it made 41 movements over 45°. Some of the lateral comparisons of the Type II larvae can be seen in the photographs.

More detailed records from a trial immediately preceding the series of photographs are shown in Table II. The measurements were taken from tracings of the actual paths of the larvae. In the table, the figures for the actual distances travelled indicate a prominent feature of the tracks that simplifies separation of larval types; namely, routes of Type II larvae are more devious than those of Type I, and those of sluggish individuals are especially devious. Consequently, the increased time taken to reach a lamp is partly a function of the greater distance travelled. Nevertheless, both the duration and the rate of travel also are affected by the amount of time spent in sweeping but hesitant lateral comparisons.

Developing Type II larvae soon begin to exhibit more precise orientation in steep gradients of light intensity than they ever exhibit in gentle gradients. This is best shown by their reactions at boundaries in dark–light alternative chambers but, even there, they behave differently from Type I larvae. Figs. 36–53 show some intra- and inter-instar differences exhibited by larvae in an alternative chamber as the temperature was raised past the level at which they modified their responses to light (12).

Figs. 36–41 show the behavior of six third-instar larvae classified as active Type II individuals. The first two figures show cluster formation at room temperature, and Fig. 38 shows the raised heads that are the standard initial response to rising temperature two or three degrees below the reaction temperature of any instar (12). As they approached their reaction temperature (Fig. 39), the larvae proceeded toward the boundary and reacted to it

without crossing but, as the temperature continued to rise, they became photonegative (Fig. 40) and would not return to the illuminated area (Fig. 41).

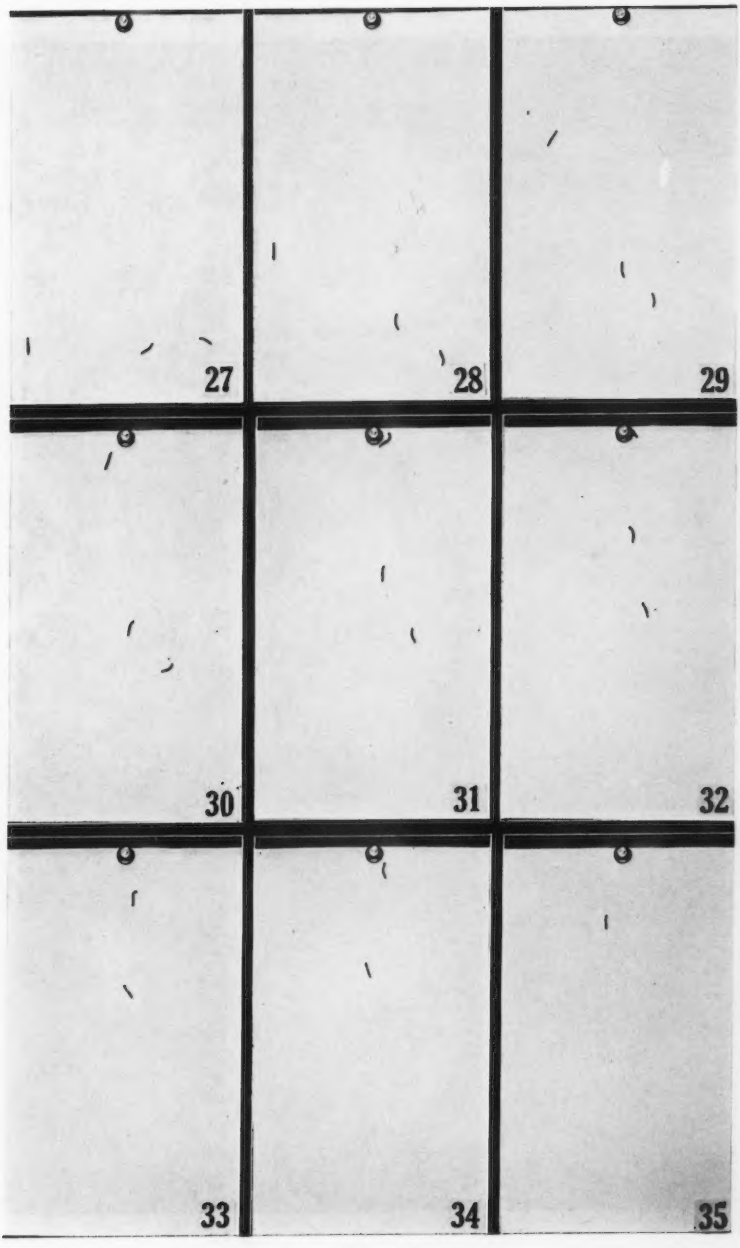
Figs. 42–47 show the behavior of six Type I third-instar larvae under similar circumstances. These larvae did not cluster, but moved about independently, making frequent contacts with the boundary even at room temperature (Figs. 42–43). As the temperature rose (Figs. 44–45), boundary contacts became more frequent, with the result that some larvae temporarily entered the dark (Fig. 45) because of more frequent boundary contacts at increased rates of travel. Finally (Figs. 46–47), the larvae reversed their orientation to light and remained in the dark.

The photographs show the greater activity and independence of Type I individuals both at room temperature and at their reversal temperature. They did not cluster, nor did they cross the boundary within the same narrow zone. Nevertheless, despite these behavior differences, both types become photonegative within the same temperature range if they are subjected to precisely similar temperature, moisture, and nutritional experiences before their tests. This explains why the two types were not identified during earlier tests in alternative chambers (12).

Figs. 48–53 show the actions of Type I fourth-instar larvae. Once again, independent movement occurred at room temperature (Figs. 48–49), and more frequent boundary contacts occurred with rising temperature (Figs. 50–51). In contrast with earlier instars, however, all types of larvae in the fourth and subsequent instars of *M. pluviale* become more *photopositive* when overheated (12), with the results shown in Figs. 52–53. As the temperature rose above their reaction point, the larvae began to cluster with their heads raised, but remained in the light. As this characteristic instar difference is important in certain field situations, it has been illustrated for future reference.

FIGS. 27–35. Contrasting behavior and activity of Type I and Type II fifth-instar larvae travelling to a 6 watt lamp in a dark room at 21° C. and 65% R.H. The Type I larva on the left was 40 mm. long, the active Type II larva in the center was 39 mm., and the sluggish Type II larva on the right was 35 mm. long. Distance between the starting line and the light approximated 58 cm. FIGS. 27, 28, and 29 at 0, 15 and 45 seconds, respectively. FIGS. 30, 31, and 32 at 58 seconds; 1 minute, 25 seconds; and 1 minute, 40 seconds; and FIGS. 33, 34, and 35 at 2 minutes, 40 seconds; 3 minutes; and 4 minutes, respectively. Photographed by electronic flash exposures. Photographs by R. Banyard.

FIGS. 36–53. Contrasting behavior of Type I and Type II third- and fourth-instar larvae in a dark–light alternative chamber as the air temperature was raised from 23° C. past 35° (instar III) and 37° C. (instar IV). Third-instar larvae (FIGS. 36–47) approximated 12 mm., and fourth-instar larvae (FIGS. 48–53) averaged 17 mm. Larvae were fed at 70% R.H. and 25° instead of 20° C. for 24 hours before the test to raise their reaction temperature sufficiently to obtain a longer series of photographs. FIGS. 36–41. Active Type II third-instar larvae forming a cluster at room temperature (FIGS. 36–37), becoming restless at 33° C. (Fig. 38), reacting to the boundary but not crossing it at 34° C. (FIG. 39), and finally becoming photonegative at 35° C. (FIGS. 40–41). FIGS. 42–47. Type I third-instar larvae moving independently at room temperature (FIGS. 42–43), and making more and more frequent independent boundary contacts at 33° (FIG. 44) and 34° C. (FIG. 45) prior to becoming photonegative at 35° C. (FIGS. 46–47). FIGS. 48–53. Type I fourth-instar larvae moving independently at room temperature (FIGS. 48–49), contacting the boundary more and more frequently between 34° and 35° C. (FIGS 50–51), and finally clustering in the light at and above their reaction temperature of 37° C. (FIGS. 52–53).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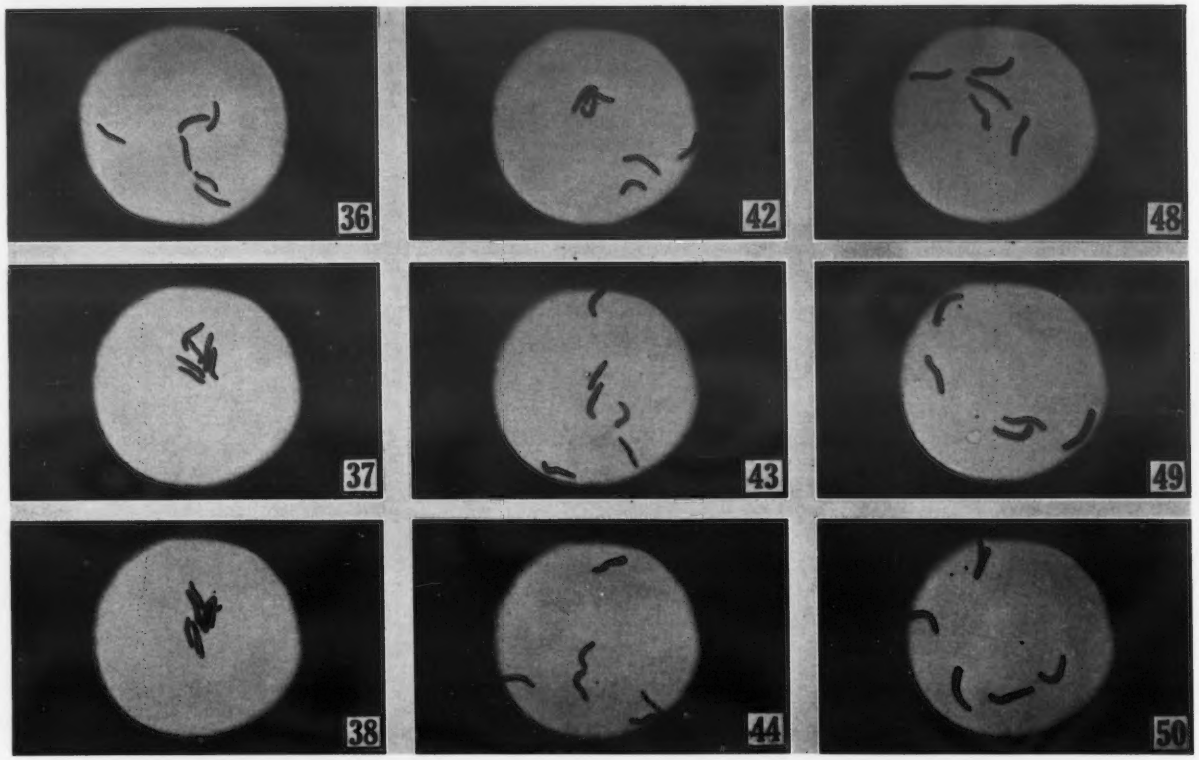
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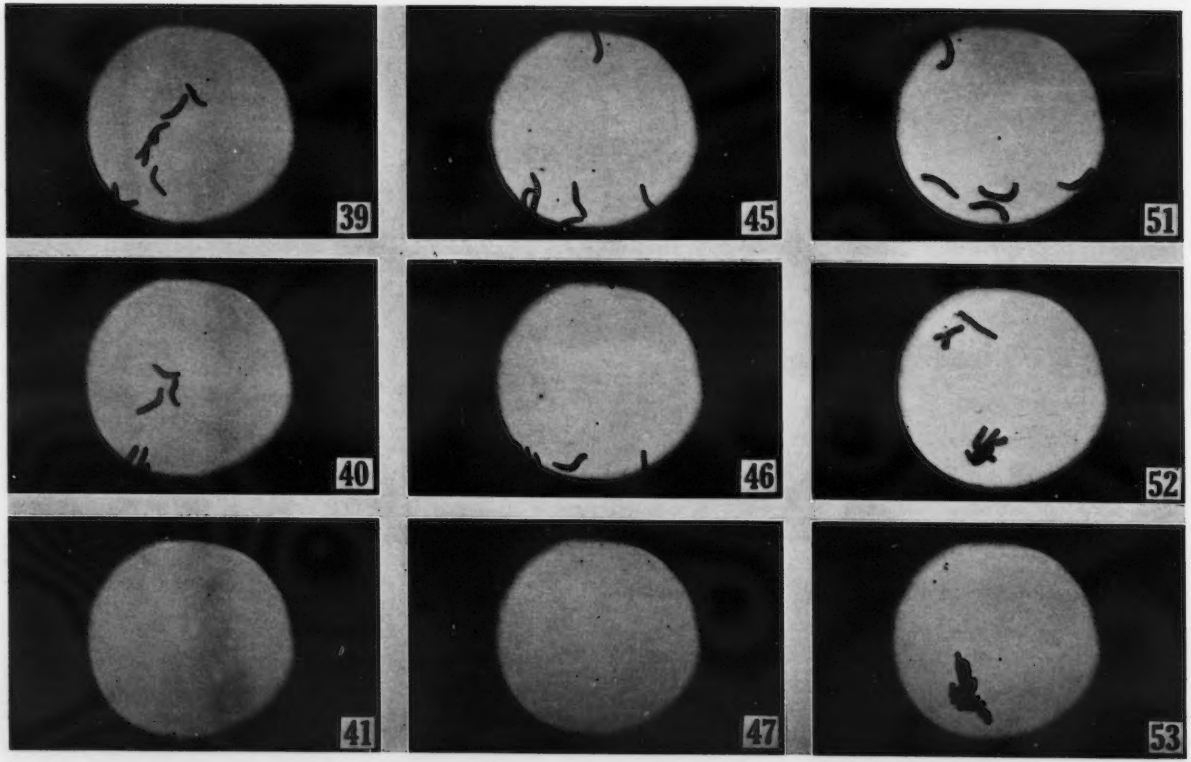

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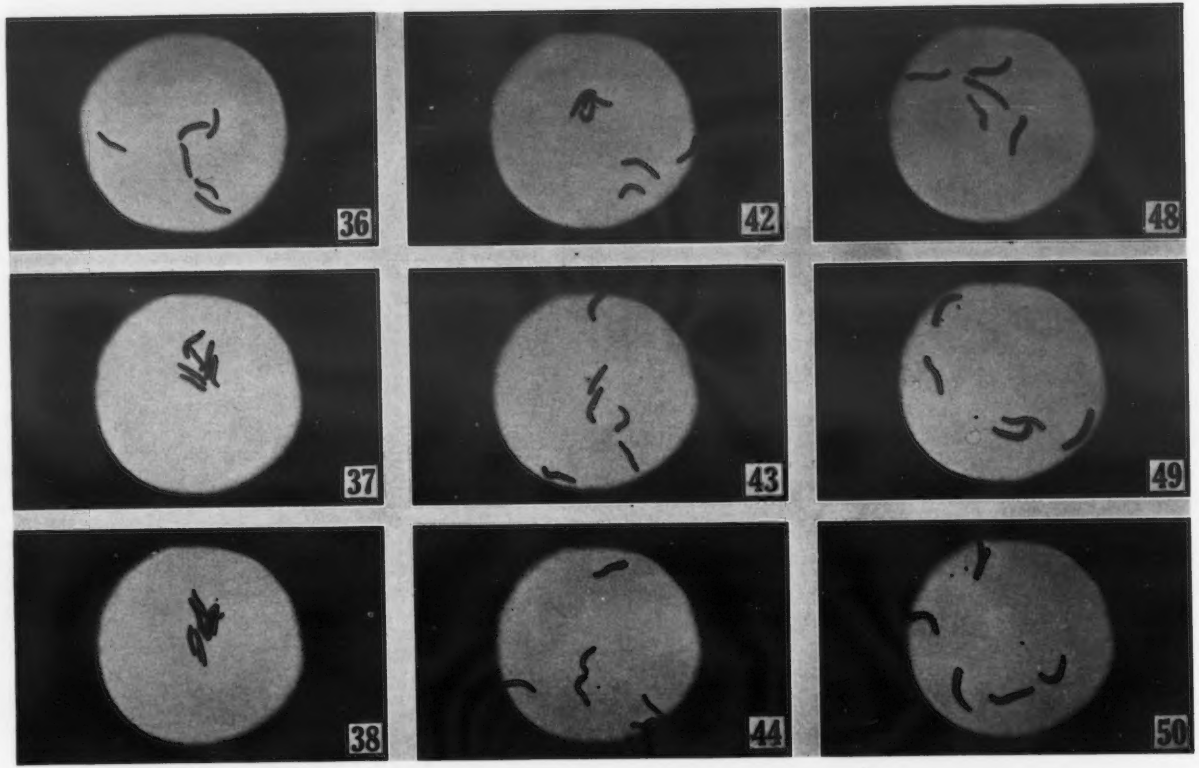
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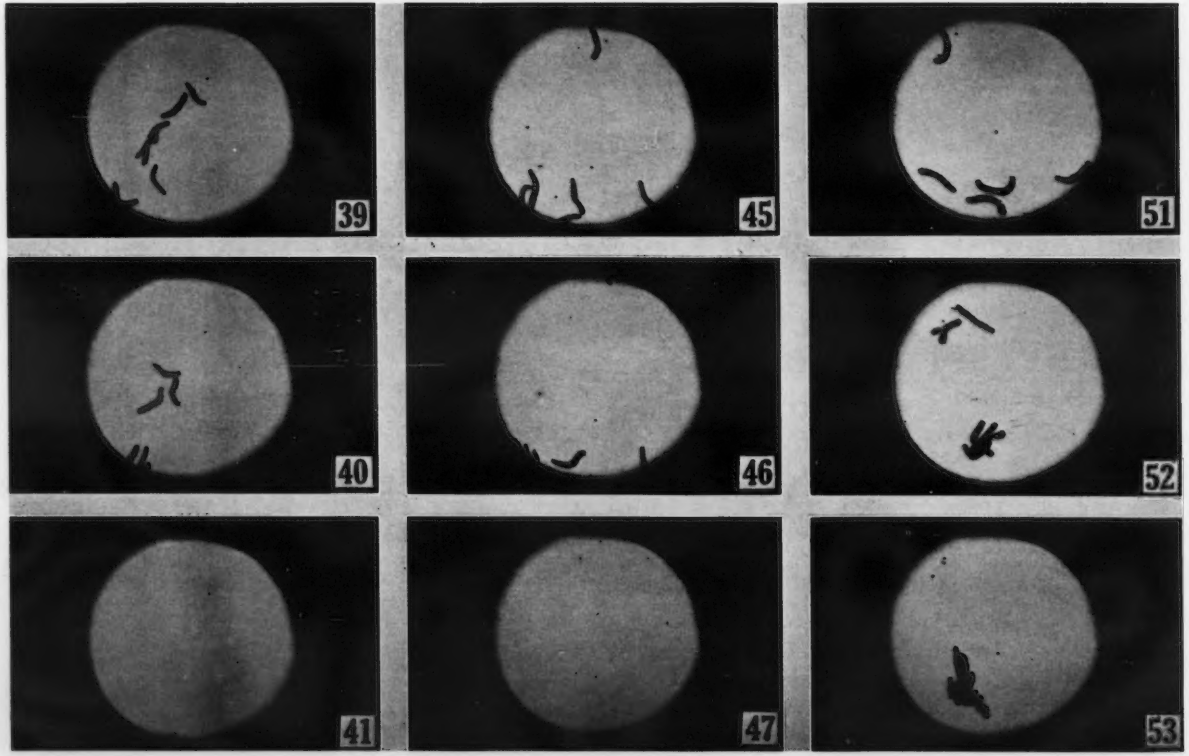

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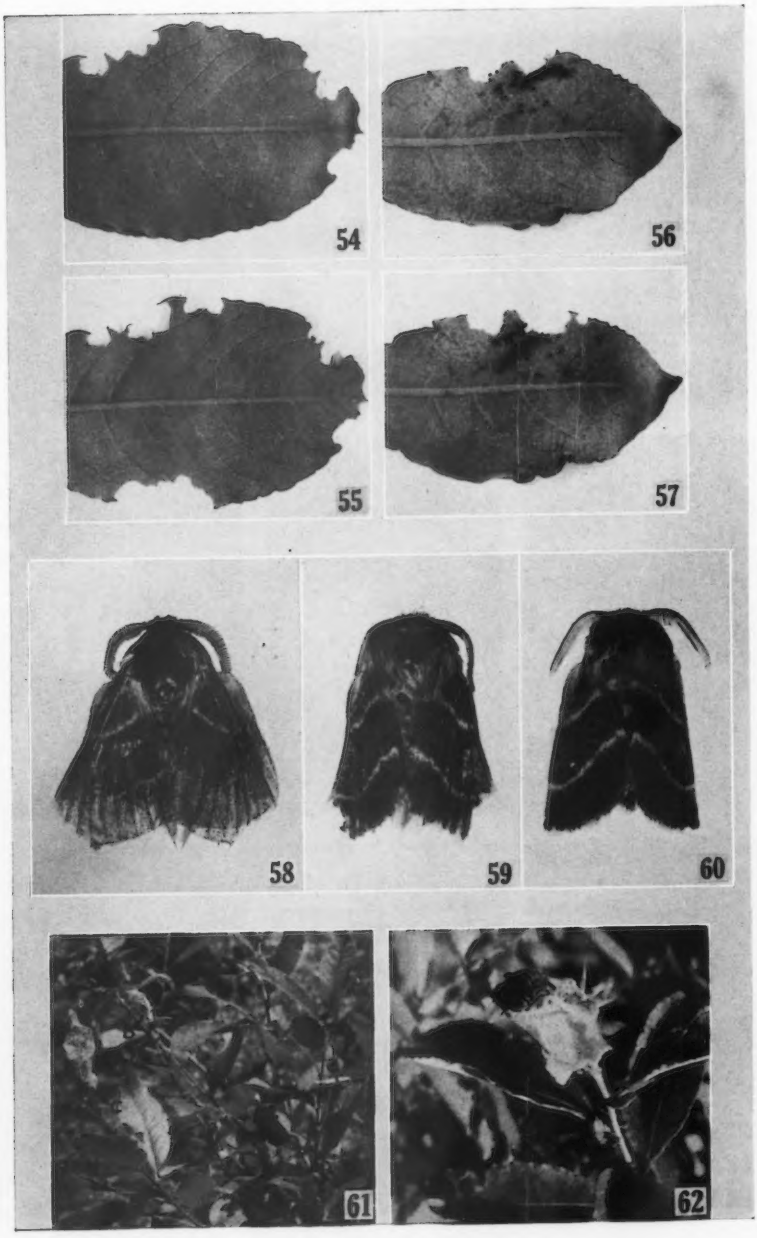
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Differences in Feeding, Development, and Survival

During development, differences in feeding behavior, rate of development, and ability to survive soon appear among the different kinds of larvae. The more fundamental differences show most clearly in individual rearings. It is difficult for any individual to survive alone during its first and second instars, but larvae of third and subsequent instars may survive, though their rates of development are slower than those of groups.

If leaves are hung vertically in jars so that much of the glass is exposed, larger Type I individuals seldom remain constantly on their food. Instead, they rest between feeding periods on a silk pad spun beside the leaf and attached to it by a bridge. They return to feed at intervals, but do not necessarily resume feeding at the last point attacked. Consequently, after 48 hours a leaf on which a Type I larva has fed may have very little silk on it in which frass can be entangled, and its edge may be chewed at widely separated points. Figs. 54–55 show 24- and 48-hour feeding on a single *Salix* leaf by a young fourth-instar Type I larva kept at 21° C. and 80% R.H.

Active Type II larvae exhibit several types of feeding behavior under comparable circumstances but, for the most part, tend to deposit more silk on their food, with consequently greater entanglement of frass particles. Sluggish Type II larvae, however, consistently rest on a leaf between feeding periods, with the result that all their silk and much of their frass remains with them. Since they move less often than other larvae, they frequently, though not invariably, feed at points within body length of their resting station during successive 24-hour periods (Figs. 56–57).

Different types of larvae consume different amounts of foliage per day. For example, in 1956, 20 Type I individuals in the first day of their third instar had an average consumption of 45 sq. mm. of *Salix* foliage, and during the first 24 hours of their fourth and fifth instars they averaged 148 and 451 sq. mm., respectively. A comparable group of active Type II larvae

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FIGS. 54–57. Feeding differences observed between Type I and Type II fourth-instar larvae over a 48 hour period. FIGS. 54–55. Twenty-four- and 48-hour feeding patterns on a 9 × 5 cm. *Salix* leaf by a Type I larva that left the leaf for each of its rest periods. FIGS. 56–57. Contrasting feeding patterns on an 8 × 4 cm. leaf given during the same period to a sluggish Type II larva that remained on the leaf during rest periods and soiled it with silk and entangled frass.

FIGS. 58–60. Degrees of battering exhibited by laboratory-reared Type I and Type II male adults left undisturbed in jars for 48 hours after emergence from their cocoons. FIG. 58. A Type I male with nearly complete loss of thoracic hair, extensive scale loss, and fraying of the forewings. Five larval instars; 262 mg. pupa; 50 days from eclosion to adult emergence. FIG. 59. An exceptionally active Type II male with moderate loss of thoracic hair and wing scales, but only minimal fraying of the wing tips. Five instars; 238 mg. pupa; 52 days from eclosion to adult emergence. FIG. 60. A typical sluggish Type II male that remained quiet without attempting flight in the emergence jar. Six instars; 208 mg. pupa; 57 days from eclosion to adult emergence. Over-all adult lengths as photographed ranged from 16 to 14 mm.

FIGS. 61–62. Differences in tents constructed in the field by Type I and Type II larvae set out about 6 weeks behind the emergence of the natural population. FIG. 61. Three of four tents constructed by a mixed group of 10 Type I and 30 active Type II larvae. Tent *a* was constructed by second- and tents *b*, *c* by third-instar larvae. The largest, *c*, was 8.8 cm. long. FIG. 62. The second and final tent constructed by 80 sluggish Type II larvae. This tent was formed during the late second instar and was enlarged until the time of the molt to fourth instar. At its largest it measured 5.5 × 4.5 cm.

consistently consumed 34% less in the third instar, and 46 and 55% less in the next two instars. Similarly, consumption by sluggish Type II larvae averaged 36% less than Type I during the third instar, 69% less in the fourth, and 58% less in the fifth instar.

Individual rates of development at 21° C. and 80% R.H. also differed. Duration of the larval stage of 40 Type I individuals averaged 36 days, whereas active Type II larvae averaged 39 days, and sluggish larvae required nearly 43 days to pupation. Moreover, Type I larvae rarely passed through more than five instars, whereas Type II larvae rarely had less than six.

These figures for foliage consumption and rate of development mean little in themselves, since they were obtained from individuals developing some 4 weeks behind field populations, and there is some evidence that they can be changed by changing the time of larval emergence in relation to the growth of the host tree, or by feeding new or old foliage consistently. Nevertheless, they are valid enough for comparative purposes. Moreover, field surveys showed that different numbers of instars also occurred in natural situations.

In group rearings in jars, advantages possessed by Type I larvae could be submerged or eliminated by manipulating rearing conditions, as indicated in the notes on establishment. Thus, groups of 30–80 active Type II larvae could be brought through their first two instars a day or two faster than 15–20 Type I larvae because of the better environment provided by their greater amount of silk. Nevertheless, such manipulations simply revealed the need for certain requirements and, when these were fulfilled, Type I larvae, alone or in groups, ate more, developed faster, and survived better than Type II larvae in jars.

Amounts and causes of mortality varied among larval types and instars in the laboratory. Desiccation and starvation of Type I and sluggish Type II first-instar larvae have been noted already. Jar rearings, however, provided unnatural circumstances. Therefore, in order to obtain information on the effects of varying the proportions of the different larval types in group rearings, 18 mixed colonies of 30–100 first-instar larvae were established on cut branches provided with water but freely exposed to the room. Each branch was set in the apex of a 30 cm. paper cone that allowed any fallen larva to make its way back up to the foliage. All groups were always placed directly on the leaves when first set out to avoid initial losses of sluggish larvae that otherwise might not locate their food. Cone rearing could be continued into the fourth instar by smearing a vaseline barrier on the cone base, but larger larvae escaped too frequently.

Dropping of all kinds of larvae from the foliage to the cones resulted in considerable first-instar mortality. Pure Type I groups experienced few losses, since larvae that fell soon returned to the foliage above. Pure groups of sluggish larvae were quickly decimated, however, since those that dropped did not survive, and the numbers remaining soon thinned to the point where they could not provide enough silk. Similarly, mixtures of Type I and sluggish Type II larvae were soon reduced to Type I survivors, since the sluggish

individuals either dropped or were left behind on a damaged, withering leaf. Furthermore, unless such mixtures contained at least 20 Type I larvae, even these did not survive past their first instar, since they seldom remained together in large enough groups to construct suitable tents. Consequently, best survival through the first instar occurred in groups of more than 20 Type I larvae, in mixed groups of Type I and active Type II larvae, or in groups composed entirely of active Type II larvae.

When groups were reared on cones, the 3 day difference in average larval span noted between Type I and active Type II larvae in individual rearings lengthened to nearly 7 days in only four instars. In other words, as long as larvae were kept directly on their food in jars, their differences in developmental rate remained small, but when they were exposed to more natural circumstances in which they had to leave tents to feed, their differences in developmental rate increased markedly. This was because the duration of the rest periods between meals varied in relation to the total activity of the colony, which in turn varied in relation to the proportions of the larval types involved.

As noted previously, rest between feeding periods seemed indispensable, but excessive amounts were detrimental. The more restless groups not only consumed more food during one feeding period but also fed more frequently, so that they grew more rapidly. The less active groups not only ate less, but did not feed often enough. In fact, sluggish groups could not be reared on cones at all, and even groups of 100 active Type II larvae spent 1.5 hours or more resting in clusters on their tent for each hour they spent moving to their food, consuming it, and returning to the tent. In contrast, groups that included 30 Type I larvae as well as 70 Type II's spent less than an hour at rest for each hour of activity. Excessive time spent in clustering was closely associated with the appearance of polyhedral virus disease for reasons noted below.

Disease had been common in field populations in previous years, so that it was naturally suspect as a factor contributing to the initial differences observed among first-instar larvae emerging in the laboratory. The possibility that there were simply sick and healthy larvae could not be overlooked. Therefore, during all types of early rearings, contamination from field and laboratory sources was avoided. It soon became clear, however, that virus, fungous, or bacterial diseases as such did not contribute to the differences. In fact, the differences affected the chances for intra- and inter-larval contamination once disease was introduced. In other words, a few individuals of any type might exhibit symptoms during early larval life, but the subsequent course of disease in individuals or in groups depended largely on the habits of the larvae and on manipulations of their environment and food.

For example, individuals of the sluggish type illustrated in Figs. 56–57 invariably died from bacterial or virus disease during their third to fifth instars if they were allowed to soil their food consistently, but they could be kept healthy if their frass was kept off their food. Similarly, disease appeared

among Type I individuals that were forced to feed consistently on food soiled by their own frass and, in these instances, it was often hastened by starvation effects, since these larvae seldom fed readily on soiled food until they were very hungry. On the other hand, Type I larvae allowed to feed like the one whose feeding patterns are shown in Figs. 54–55 did not exhibit disease symptoms unless they were fed leaves known to be contaminated.

On cones, Type I larvae or mixtures of Type I and active Type II larvae shifted their feeding sites frequently and constructed several tents during their first two instars. Consequently, any ailing individual was left behind to die, and its contaminated remains were out of contact with the other larvae. Chances for interlarval contamination in such groups increased sharply if the air temperature was raised to 30° C. or more. This wilted the cut foliage, and the resulting increased wandering during incipient starvation provided continual contacts between disease-free but weakened larvae and infected individuals or their frass.

On the other hand, pure Type II groups shifted feeding sites less frequently, so that they often fed on leaves continually attacked by other individuals. They also tended to enlarge their original tent instead of constructing others. Moreover, as noted, they spent longer intervals resting in clusters on the tent, since they lacked stimulation by the more restless Type I larvae. These differences soon resulted in different amounts of disease. For example, groups that clustered for 1.5 hours or more suddenly succumbed to virus disease in the third instar, whereas their more active companion groups did not suffer until their members were confined to jars in which they were forced into continual contact with food contaminated with frass and old silk.

When larvae cluster only briefly on a tent, their movements before and after clustering produce extra layers of silk in which any frass pellets dropped before or during the rest period are quickly buried. Prolongation of the clustering period, however, leaves accumulating frass exposed, so that chances for infection from this source must be added to those for infection from dying larvae in prolonged contact with others in the cluster. In addition, when digestion is prolonged, there are evidently physiological changes, since larvae that cluster for long periods are consistently more flaccid than those that feed frequently, and their regurgitated juices are darker. Such individuals appear to be less resistant to infection over a period of time than those that feed more often (cf. 5, 6), but they do not die from disease if they are kept from sources of infection.

During early May, field populations remained colonial and engaged in little intertree wandering. Therefore, it was possible to collect foliage for laboratory stocks from trees free from tent caterpillars. During this period, virus disease in the laboratory occurred sporadically in a few individuals from some egg masses, in which it was apparently latent. Although it was impossible to predict where disease might appear, it was always possible to predict its future course in undisturbed or in manipulated groups because of the differences described above. Later in the season, during a 3 week period when larvae

were wandering in the field, no tree was free from tent caterpillars, their damage, or their frass. During this period, individuals or groups fed on field-damaged leaves suddenly died from polyhedral virus. In fact, disease could be kept out of later rearings only by selecting *Salix* leaves expanding after the field populations pupated.

Another important cause of mortality was apparently innate physiological weakness that appeared most frequently among sluggish larvae during the first 24 hours after emergence. Whole groups of 30–70 sluggish larvae from some egg masses died after little or no feeding. They contained no signs of virus, fungous, or bacterial diseases. Moreover, other members of these egg masses showed no more signs of latent disease during their first two instars than those from other families. In later instars, inability to begin or complete ecdysis was common among sluggish larvae, accounting for all deaths but those from disease in rearings where starvation was not a factor. It occurred less often among active Type II larvae, and only rarely among Type I individuals.

Differences Among Laboratory-reared Adults

Few adults were obtained in the laboratory, since stocks were used for experimental purposes, but moths that were available were used to develop techniques for studying field-collected material. Moths obtained from known types of larvae were tested for spontaneous activity during the first 48 hours after their emergence. Information on their tendency to attempt flight while undisturbed was required. Therefore, they were not tested on flight mills because of the undue stimulation involved. Instead, they were allowed to remain undisturbed in the jars in which they emerged. If they were active, they attempted to fly and battered themselves to varying degrees during the 48 hour period. If they were sluggish, they seldom moved, so that they retained their scales. Figs. 58–60 show the three broad groups that could be distinguished among laboratory-reared individuals kept at 23° C. and 65% R.H. after emergence fom the cocoon.

After 48 hours Type I adults appeared much like the male in Fig. 58 and even in 24 hours they were frequently worse than the Type II male shown in Fig. 59. The thorax was worn smooth, most of the wing scales were lost, and the edges of the forewings were tattered. Type II individuals after 48 hours sometimes were active enough to batter themselves to the extent shown in Fig. 59, but more often they approximated Fig. 60 more closely in appearance. Sluggish individuals that emerged successfully remained as perfect as the male in Fig. 60.

Differences Among Artificial Colonies Outdoors

The cone rearings had indicated that different types of larvae might construct different numbers of tents, but the limited space they provided made it difficult to assess differences in tent shape that frequently occurred. Consequently, 23 experimental colonies totalling some 1700 late first- and early

second-instar larvae of similar age were set out on closely spaced small *Salix* trees where their behavior could be followed.

The colonies were set out directly on leaves nearly 6 weeks later than the first emergence of the natural population, so that they had different foliage size and different types of predators to deal with. Moreover, several days of cold, wet weather occurred soon after they were set out. Nevertheless, since the larvae were near their second instar their establishment losses were low. On the other hand, differences observed in the laboratory were magnified.

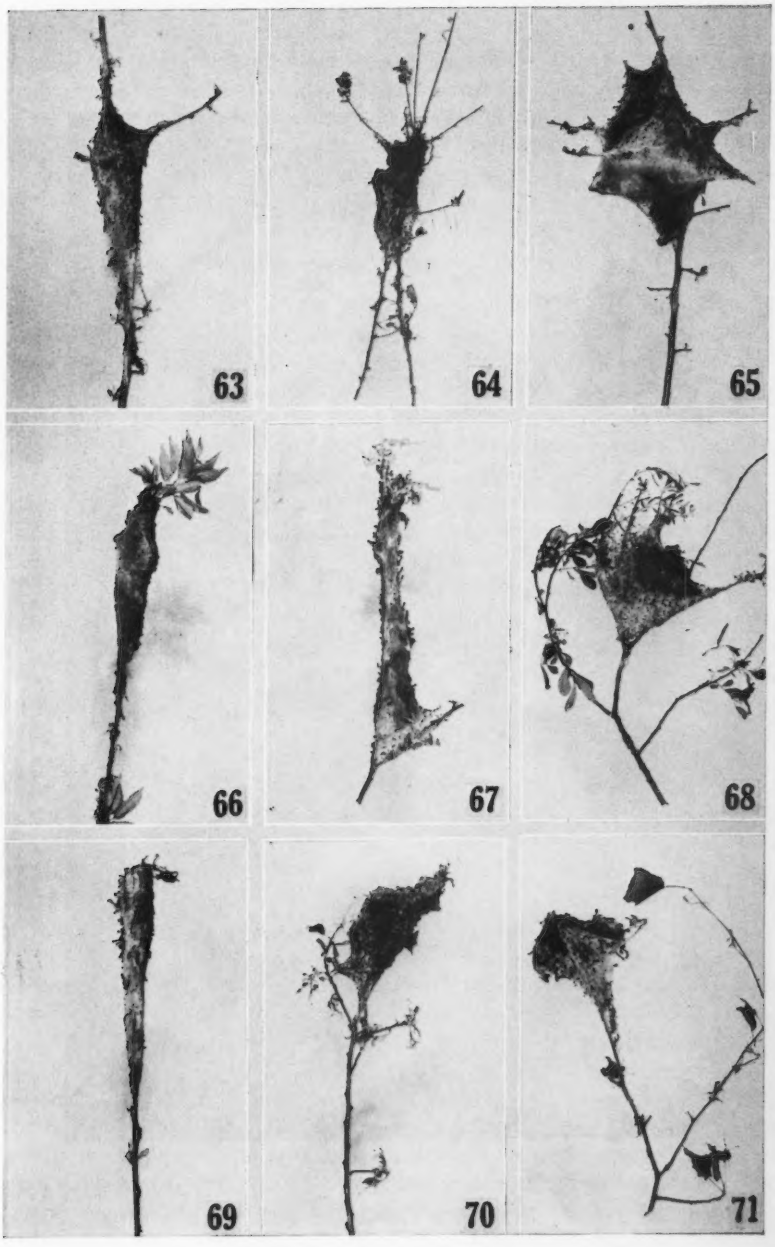
For example, pure Type I colonies not only constructed several tents in a relatively short time but they spaced these and their feeding sites over long distances that involved very extensive travel for such small larvae. Thus, one colony of 80 Type I larvae constructed seven tents in 4 days and fed on 10 leaves scattered over a meter of twigs. Because of inclement weather, they and colonies like them took 16 days to pass through their second instar and into the first part of the third. Moreover, during their extensive travels, groups from such colonies frequently fell prey to spiders, which were comparatively large by that part of the season. Nevertheless, their high level of activity brought them out of rain-induced clusters as soon as the rain stopped, so that they took full advantage of favorable weather.

In contrast, a colony of 80 sluggish Type II larvae comparably exposed on an adjacent tree constructed one tent and remained feeding on one leaf for more than 10 days. They remained on the leaf after their feeding had cut some of the main veins and withered half the surface. Six days later, when the leaf withered completely, the survivors moved 50 cm. to new leaves and constructed their second and final tent. At this time they were still in their second instar, since they lost many opportunities for feeding by remaining clustered during favorable weather.

Such differences were not exceptional. Colonies composed of sluggish larvae or mixtures of sluggish and active Type II larvae seldom constructed more than one tent during four instars. Where possible, they continued to feed on leaves close to it, with the result that much of their feeding took place on previously damaged leaves. Pure groups of active Type II larvae were not very different. Colonies containing 20% or more Type I larvae in addition to active Type II larvae, however, ranged almost as widely as pure Type I colonies, so that they fed more often on undamaged leaves. They also constructed two or more tents during four instars.

The most sluggish colony that survived to the fourth instar was the group of sluggish Type II larvae described above. Its surviving members died from disease while they were molting. Fig. 62 shows the larvae clustered on their tent 24 hours before their death. Fig. 61 shows three tents made by a mixed

FIGS. 63–71. Types of tents constructed by *M. pluviale* colonies. FIGS. 63–65: Tents formed on branches or stems. Remaining figures: tents formed at or near branch-tips. FIGS. 65, 68, and 71: compact tents. Remaining figures: elongate tents. Dimensions (greatest length and width): FIG. 63, 27.5 × 7.8 cm.; FIG. 64, 15 × 6.2 cm.; FIG. 65, 17.5 × 14 cm.; FIG. 66, 18.8 × 5 cm.; FIG. 67, 32.5 × 6.8 cm.; FIG. 68, 10 × 7.5 cm.; FIG. 69, 22.5 × 3 cm.; FIG. 70, 16.3 × 6 cm.; FIG. 71, 6.3 × 5.6 cm. (exclusive of 3.5 cm. silk sheath on twig). Photographs by R. Banyard.



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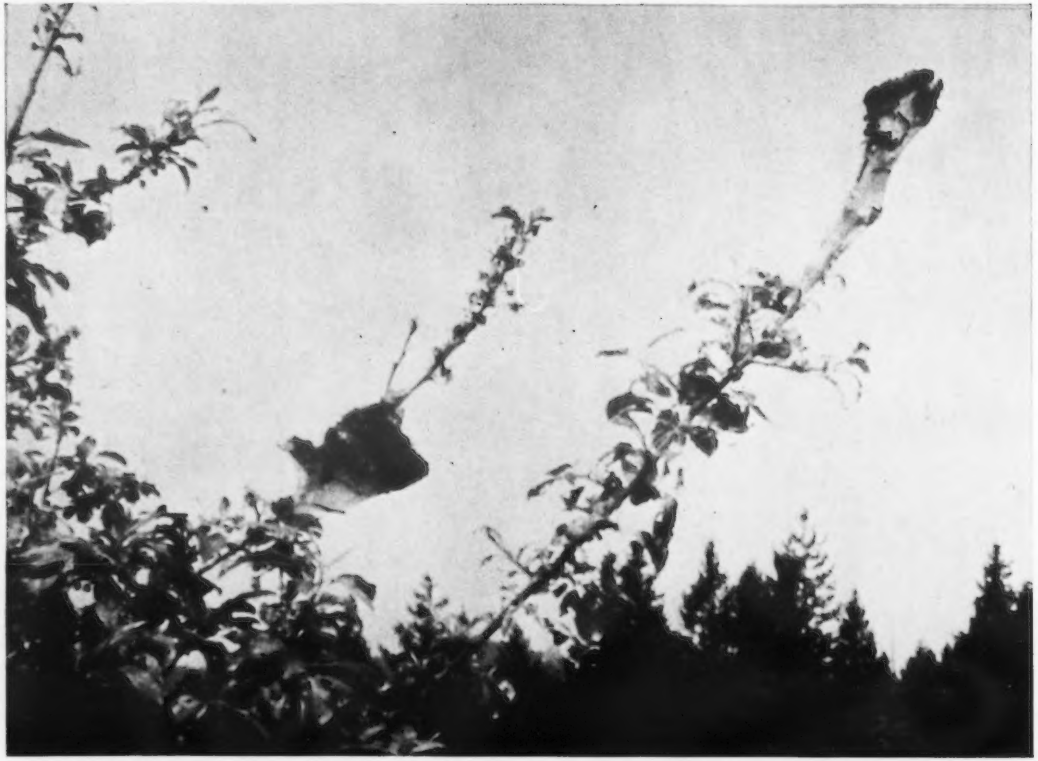

FIG. 72. Compact and elongate tents formed by two separate *M. pluviale* colonies occupying adjacent branches of a roadside apple tree. Stages of development in the whole colony of larvae clustering on the compact tent ranged from early third to midfourth instar, but the majority were approaching their molt to the fourth instar. The comparatively few larvae clustering on the elongate tent were molting to the fifth instar, but the bulk of the colony feeding away from the tent ranged from half-grown fourth- to young fifth-instar larvae.

group of Type I and active Type II larvae that left the tree during their fifth instar 7 days before the larvae in Fig. 62 even began to molt to their fourth instar.

Within the limitations imposed by the large leaves, tents constructed by different types of colonies took on different shapes foreshadowed by laboratory colonies on cones. Tents constructed by relatively inactive colonies had nearly equal sides (Fig. 62), so that they were compact. In contrast, tents constructed by active colonies were longer than wide (Fig. 61) and often were nearly cylindrical or clavate.

Differences Among Natural Colonies

Laboratory and outdoor observations established the more obvious differences among the different types of larvae originally separated by a simple test after their eclosion. They also indicated that groups containing different proportions of these types could develop at different rates and have different chances for survival. Since the types were stable, it was clear that natural populations might contain different proportions of them in particular circumstances. Therefore, natural colonies were examined in some detail, using differences in tent shape as a guide. Figs. 63–71 show the first results.

Tents constructed by natural colonies may be classified in two groups: those that occur well back on a branch or at a fork in the branch or stem (Figs. 63–65), and those that occur near the tips of twigs (Figs. 66–71). Both categories may be divided readily into compact (Figs. 65, 68, 71) and elongate types, as illustrated in the series of photographs. In the compact type, the bulk of the tent consists of nearly equal surfaces, so that the structure is roughly pyramidal or box-like. The other figures show that variations of the elongate type are consistently more than twice as long as they are wide, and often more than three times longer.

Examination of the habits and general development of colonies associated with the different types of tents showed differences in feeding pattern and, in comparable exposures, differences of up to an instar in average stage of development. The two colonies illustrated in Fig. 72 provide the best single example, because they occurred side by side on adjacent branches of the same apple tree, but had not mingled.

The photograph was taken May 13, 33 days after the first emergence occurred in the area. Larvae associated with the elongate tent in the upper right of the photograph ranged from half-grown fourth- to young fifth-instar individuals. The few clustered on the tent were mature fourths beginning to molt. Most of the colony was feeding over 5 m. of main branches and a larger amount of associated laterals. Damage occurred sporadically over the whole distance. The feeding highways were not connected with the branch on which the compact tent was located, but they were connected with two smaller, abandoned tents near the opposite end of the 5 m. distance. The egg mass was attached to a twig 1 m. from the smallest tent and 4 m. from the tent illustrated.

The whole colony associated with the compact tent on the left was clustered on it when the photograph was taken. They ranged in development from young third-instar larvae to a few half-grown fourths. Most appeared to be molting to the fourth instar, but larvae of all the stages noted were resting quietly in the cluster. Their total feeding distance extended less than 1 m. back from the branch tip, with the result that most of the leaves had been nearly consumed, whereas those that were not were heavily silked. The tent shown was the only one that had been constructed. It consisted of several layers, and the egg mass was embedded in the silk at its basal end. It is worth noting that in both instances the opposite type of tent could have been formed, so that the difference in shape was not a limitation imposed by tree structure at different points.

Such differences were common. In addition, it was easy to locate starving larvae as much as two instars younger than the colony average lingering in smaller elongate tents abandoned by active, roving colonies. In most instances, 1 to 10 such larvae could be found, and sometimes the dried remains of one or two diseased individuals appeared on the surface of the old tent. Moreover, on particularly compact tents, most of the colonies remained clustered for several days at a time during weather in which active colonies were ranging widely on feeding excursions. There was no doubt, therefore, that different types of colonies occurred in natural situations, but it was difficult to detect much order within the outbreak until previous estimates of population density were consulted.

Differences Among Infestations

In 1955, a roadside survey of the outbreak located areas of heavy, medium, and light infestations in terms of numbers of tents per tree. Most trees were less than 7 m. high, so that larvae in the fourth instar frequently overlapped feeding areas if there were more than 10 tents per tree. Therefore, the tentative scale was: light, 0–4; medium, 5–10, and heavy, >10 tents per tree. This proved to be a reasonable approximation in view of the subsequent discovery that colonies were capable of constructing more than one tent. Furthermore, it entailed little confusion of borderline cases, because areas that contained more than 10 tents per tree usually included many trees that had 20–50 tents each. Consequently, in all but a few localities, the boundaries were well defined. Therefore, in 1956 it was possible to survey some of the areas to determine not only the current status of their populations but also the proportions of the different types of tents they contained. Sample counts were made in localities previously classified as free, light, medium, and heavy in 1955, with the results shown in Table III.

These results showed very clearly that initial infestations consisted largely of colonies that were active enough to form elongate instead of compact tents, whereas the heaviest, i.e., the *oldest* infestations contained a much larger proportion of colonies that formed compact tents. In other words, most of the egg masses deposited in previously uninfested areas apparently yielded

TABLE III

TENT CATERPILLAR INFESTATION LEVELS IN 1955 AND THE NUMBERS OF ELONGATE AND COMPACT TENTS FORMED IN THE SAME AREAS IN 1956

	Numbers of elongate tents in 1956	Numbers of compact tents in 1956	Totals
Areas with no infestation in 1955[a]	488 (324.715)	71 (234.285)	559
Areas with light infestations in 1955[a]	429 (400.811)	261 (289.189)	690
Areas with medium to heavy infestations in 1955[a]	487 (678.474)	681 (489.526)	1168
	1404	1013	2417
$\chi^2 = 329.571$; $P < 0.001$			

[a]Absent: 7 areas, 87 trees sampled in 1956; light: 5 areas, 50 trees sampled; medium to heavy: 7 areas, 78 trees sampled.

good percentages of Type I larvae, whereas most egg masses deposited where the population had been increasing for a few years apparently yielded higher proportions of Type II larvae per colony. During 1956, this could not be checked directly, because intertree travel and colony mingling began to interfere, so that many colonies could no longer be associated with a particular egg mass with any certainty. Nevertheless, an indirect check was possible.

Since there seemed to be a preponderance of active colonies in initial infestations and an excess of less active colonies in older infestations, it seemed reasonable to suspect differences in adult activity. Eggs can only be deposited in previously free areas by females that fly or are blown there. Therefore, it appeared that any less active adults might contribute most heavily to infestations in which they emerged, whereas more active adults might be capable of ovipositing there and in more distant, previously uninfested areas as well. To check this in the field, however, a very detailed survey to locate sharp divisions between types of infestations was necessary.

Fortunately, nearly 7 mi. of road surveyed in 1955 provided the ideal situation, since the strip had been nearly free from tent caterpillars, and the locations of the few 1955 colonies near each end were known exactly. In 1956, many of the roadside trees were moderately infested, so that their positions could be plotted and the numbers and types of tents they contained could be recorded. The results are summarized in Table IV.

TABLE IV

DISPERSAL OF *M. pluviale* ALONG A PREVIOUSLY UNINFESTED ROADSIDE

	Intervals[a]					
	Mi. 0–1.1	Mi. 1.1–1.3	Mi. 1.3–3.5	Mi. 3.5–3.9	Mi. 3.9–6.7	Totals
No. infested trees, 1955	2	0	0	0	5	7
No. infested trees, 1956	41	6	54	18	188	307
No. elongate tents, 1956	74	11	76	54	497	712
No. compact tents, 1956	23	4	0	14	135	176
Max. distance between a 1955 tent and a 1956 compact tent	0.2 mi.		—	0.5 mi.		—

[a]Note changing distances between intervals.

The records in Table IV show that colonies capable of forming elongate tents closed the previous 2.8 mi. gap completely, whereas colonies that formed only compact tents did not appear farther than 0.5 mi. from the site of a 1955 colony. It is worth noting that the 0.5 mi. record was made in the direction of the prevailing wind, whereas the 0.2 mi. distance was upwind. This evidence, together with that from the previous table, implicated differences in adult activity strongly enough for their inclusion in any working hypothesis.

On the other hand, there are some parts of the table that should not be taken too literally. It is unwise to assume that eight 1955 colonies on seven trees were responsible for 888, or even 307 new colonies in 1956. Apart from the fact that some 1956 colonies must have formed more than one tent before the count, there had been other 1955 colonies a mile or so farther on past mile 6.7. Consequently, if one admits that active adults were capable of closing at least half of a 2.8 mi. gap, one should also be prepared to admit that they might close another gap and contribute to the 1956 infestation between mile 3.9 and mile 6.7. The records show, in fact, that mile 5.7–6.7 contained 249 elongate tents, whereas mile 0–1.0 contained only 69. Since there were three 1955 tents involved in the former instance and two in the latter, it is probable that some of the 249 tents formed in 1956 were produced by colonies introduced into mile 5.7–6.7 from the more distant infestation.

Pupal collections were made in areas in which tent types had been classified and where some of the history of the infestation was known. The emerging adults were treated similarly to those reared from laboratory stock, and their spontaneous activity was classified according to the same scale. It was necessary to add an additional category for some field-collected males that were active enough to shred their wings in 48 hours. The data were grouped so that the sluggish category included males with little or no scale loss and no more than two minor breaks in the wing edges. The active category included males with moderate to extensive scale loss and frayed to completely tattered wings. Females were always less active than males just after emergence, so that they were classified as active if they showed any signs of wing damage. Table V shows the distribution of activity for both sexes in relation to the status of the infestations in 1956.

TABLE V

SPONTANEOUS ACTIVITY OF ADULTS IN RELATION TO INFESTATION AGE

	Adult type		
	Sluggish	Active	Totals
Infestation age in 1956			
First year[a]	0 (10.047)	44 (33.953)	44
>One year[a]	29 (18.953)	54 (64.047)	83
	29	98	127
$\chi^2_c = 17.989$; $P < 0.001$			

[a]Five new areas; four old areas.

The activity differences shown in Table V are in general agreement with preceding results, but they show no trace of the few sluggish individuals that might be expected to appear by the time adults emerge in a new infestation. The small numbers involved might be partly responsible for this discrepancy, but it is more probable that any sluggish individuals in these sparse populations lagged far enough behind the average rate of development to escape collection. These laggards might have been obtained by a special collection timed to coincide with final pupation in the areas, but heavy parasitism throughout the 1956 outbreak area indicated little could be expected from such methods. Therefore, the problem was approached in a different way.

Collections of pupae had been obtained from different types of infestations to determine percentages of parasites and moths emerging in different localities. Consequently, these collections could be grouped with reference to infestation age and distance from other sources. Table VI shows the results when the new 1956 infestations were classified with reference to their distance from older infestations and examined not only for percentages of emerging parasites and moths but also for percentages of individuals that did not complete pupation successfully but were apparently unparasitized.

The results in Table VI show first a marked difference in "unexplained" mortality between two broad groups of infestations: those that were new in 1956 but more than 0.5 mi. distant from older infestations, and those that were new but closer to old populations or else were old and extensive. Although this mortality is unexplained in one sense, the preceding results suggest that it is largely a measure of the vigor of two types of populations. Attrition of colonies through mortality of sluggish Type II individuals throughout the larval period has already been indicated, and it seems reasonable to accept the results in Table VI as a measure of the final portion of this mortality before the adult stage. In addition, it is probable that the difference in successful parasitism also is simply a reflection of differences in numbers of parasitized larvae incapable of living long enough for the parasites to complete their growth. Most of the parasites of *M. pluviale* in 1956 were highly motile Diptera distributed throughout the outbreak area, and there was no evidence

TABLE VI

UNEXPLAINED MORTALITY, SUCCESSFUL PARASITISM, AND ADULT EMERGENCE IN PUPAL COLLECTIONS FROM *M. pluviale* INFESTATIONS DIFFERING IN AGE AND DEGREE OF SEPARATION

	Infestation type			
	New,[a] >1 mi. from old infestations (3 areas)	New, [a]interspersed among older infestations at 0.5–1 mi. intervals (3 areas)	New, [a]<0.5 mi. from older infestations (4 areas)	Old, for distances >1 mi. (4 areas)
Total pupae collected	140	300	63	379
% dead, unparasitized pupae	10.7	12.3	26.9	24.8
% pupae with emerging parasites	50.7	50.0	42.9	44.9
% emerging adults	38.6	37.7	30.2	30.3

[a]New in 1956.

that they were any less active in one locality than in another. Therefore, the differences in the table may well be reflections of differences among the host insects.

The Course of the 1956 Outbreak

On April 7, 1956, a protracted period of mild weather began in the Saanich Peninsula of Vancouver Island. By April 11, eclosion began in the field, but did not become general until nearly a week later. From April 7 to May 5, official weather records showed no measurable precipitation, and the period was classified as a protracted drought. Nevertheless, in the outbreak area during that 29 days there were 7 days with some rain and 11 days with broken or recurrently overcast skies. In other words, weather was ideal for the first part of larval development, with sufficient sunlight to provide radiant heat in mild air temperatures, and sufficient, but not excessive rain and dew. Consequently, development was rapid, and the more active colonies entered their second instar by April 24.

From May 5–13, the weather remained favorable, so that active colonies began to enter their fifth instar by May 11, though sluggish colonies ranged between second and fourth instars even in favorable exposures. In cool, shady locations, some retarded colonies were still in their first instar on May 13.

May 14 marked the beginning of a 5 day heat wave that had disastrous results. Prior to that date, development of active larvae had been so rapid that a few had progressed sufficiently into the fifth instar to leave the colonies for brief solitary existence prior to pupation even before any adult parasites appeared. The majority of the active colonies, however, were still within a day or two of disbanding when the continuous heat began. Figs. 52–53 showed the standard response of overheated older larvae, and this occurred in all colonies throughout the peninsula. For 5 days, fourth- and young fifth-instar larvae remained clustered in masses on the sunlit sides of the tents, trapped throughout the daylight hours by their photic response to overheating. In ordinary circumstances, this response has definite survival value, since it brings larvae out of overheated tents into relatively cooler air outside (12). During ordinary days, however, the temperature soon drops and the larvae do not remain clustered but move off to their feeding sites. In contrast, during the heat wave, larvae remained clustered for more than 12 hours at a time. Some feeding occurred through parts of the first two nights, but eventually larvae were weakened to the point where they remained clustered for nearly 24 hours per day. During the same period, smaller larvae in the sluggish colonies fared no better, since they became photonegative and clustered under their tents in any available shade.

This period had several results. Practically no growth occurred, since there was very little feeding. Consequently, fifth-instar larvae that had been about to leave the tents did not begin to do so until May 19, when they were released by cooler weather. The fact that they were weakened probably

had serious consequences, but more immediate hazards came from a wave of parasitism, chiefly by the fly *Tachinomyia similis* (Will.), which began to appear in numbers by May 16. These parasites had ideal opportunities for maximum oviposition, because thousands of colonies in the fourth and fifth instars were clustered passively together on their tents. Consequently, by the end of the period, it was difficult to find fifth- or old fourth-instar larvae without parasite eggs attached to them.

In addition to providing unusual opportunities for parasites, the weather provided ideal circumstances for an upsurge of polyhedral virus. As noted previously, prolonged clustering at even moderate temperatures has detrimental effects. In the field, disease that had been very sparsely distributed amongst even sluggish colonies suddenly became prominent, though its full effects were delayed until the larvae began to feed again.

When the heat wave broke, prepupational travel soon became general. In fact, some pupation was noted as early as May 26, though this was probably by individuals that had left the tents just prior to the heat wave and so escaped its more serious consequences. Indeed, this small amount of pupation was followed by a 6 day gap, after which pupation became more general among members of more active colonies. During the prepupational wandering, however, there was continual intermingling of diseased and healthy larvae. In addition, in more densely populated areas, active colonies of late fourth- and early fifth-instar larvae that starved during the heat wave foraged farther than usual, with the result that they often entered diseased colonies temporarily before returning to their own tents. Such mixing spread the disease not only among active mature larvae but also from sluggish third- to active fourth-instar larvae.

June became progressively wetter and colder, so that sluggish colonies were even more retarded in development, providing fresh material for parasitism as the latter part of their fourth instar was prolonged. In fact, though the second wave of pupation was at its peak in mid-June, there were still many colonies of relatively sluggish fourth-instar larvae in evidence in favorable locations. Few of these even entered the fifth instar, since disease, parasites, or simple starvation destroyed them. The wet, cool weather prolonged the pupal period, however, and many pupae fell prey to small mammals and insects, notably the European earwig, *Forficula auricularia* L. A sparse flight from the June pupation occurred near the end of the first week in July, but the fate of the portion of the population that pupated in late May could not be determined.

Functions, Advantages, and Disadvantages of the Different Types of Individuals in Colonial Life

During the larval stage, Type I individuals have several important functions within the colony that are based on their higher level of activity and their greater abilities to orientate precisely and to respond quickly to changing conditions. In the first instar they provide direction to the otherwise largely

undirected activity of the emerging larvae and, in some instances, are wholly responsible for the establishment of a colony on its food. Throughout the first four instars their higher level of activity keeps colonies in which they are plentiful restless enough to feed regularly and construct several tents. Moreover, they establish a larger network of highways and open up new feeding areas, with the result that colonies stirred to activity have more chances to encounter undamaged food. Therefore, in colonies well supplied with such larvae, development proceeds rapidly during favorable weather, chances for infection remain few when trees are not densely populated, and, in ordinary circumstances, there are good chances for colony dispersal before parasite attacks become frequent. Finally, there is good evidence that well-fed, vigorous Type I adults are largely responsible for relieving some of the population pressure in original foci by dispersing some of the next generation to more distant areas.

On the other hand, some of the Type I qualities can be less desirable from the standpoint of colonial life. Their proclivity to wander may result in their own death by predation or accident or else in the loss of groups that they lead within range of predators such as ants or spiders. Therefore, in years when such predators are plentiful during earlier larval instars, undue concentrations of Type I individuals could increase colony losses. Furthermore, in later instars on densely populated trees, wandering too far afield often leads to contact with disease and its introduction into colonies previously free from it. In addition, well-nigh pure groups of Type I larvae that might exist in natural situations would be quite capable of splitting into so many subgroups that none would be numerous enough to construct suitable tents for protection against desiccation and predation.

Active Type II larvae in mixed colonies that contain sufficient Type I larvae fulfill two valuable functions. Their tendency to group in relatively small areas results in more closely woven silk wherever they act together, so that colony tents to which they contribute are tougher and less permeable to water droplets and vapor. In addition, some of these larvae are always available as clustering foci during the rest periods required by all types of individuals. Without such foci, Type I larvae occasionally remain too restless between feeding periods, so that they may be exposed to additional water loss. Tightly packed clusters protect larvae in them from excessive evaporation during the rest periods.

On the other hand, active Type II larvae left to their own devices tend to remain clustered too long, which slows their rate of development and, in some situations, increases their chances for infection by disease or for concentrated parasitism. When they do become active, they tend to remain too close to the tent to encounter undamaged food. This may not be so important on trees like *Crataegus* or *Salix*, where there are many leaves within short distances, but on *Alnus* or other genera in which leaves are widely separated, foliage near the tent is soon reduced to very poor quality. Even so, Type II larvae by themselves seldom travel far beyond it, so that their development suffers.

Such semistarvation in the midst of plenty occurred too often in the field to be coincidental, so that it is a factor to be reckoned with in tent caterpillar population dynamics.

There appear to be few advantages for a colony that contains a high proportion of sluggish Type II larvae. In some instances, most of them die before the end of the first instar, leaving too few survivors of other types for successful development. In other instances, sluggish larvae that are capable of surviving past the first instar may be dangerous to the health of relatively inactive colonies if they contract disease within the single tent. On the other hand, those that are vigorous enough to survive to the fourth instar but cannot complete pupation have more interesting possibilities, since they may act as traps that waste the potential of ovipositing parasites. This was observed in some localities in 1956, and it is worth considering further.

The preceding section included a description of the delaying effects of a heat wave on larval development and of the results when parasites caught the colonies resting passively on their tents. Apparently, developmental rates of host and parasites are not invariably synchronized, since only 2 more days would have permitted the majority of the Type I larvae in favorable exposures to leave the colonies and escape *Tachinomyia*. In 1956, the actions of *Tachinomyia* showed that it is more attracted to clusters than to isolated larvae, though there is no doubt that it will attack individuals if it encounters them. Therefore, in some seasons it is probable that less active larvae, including many sluggish individuals not destined to become adults in any case, would be apt to absorb a percentage of the egg complement of such parasites. How far-reaching the effects of such wastage might be is problematical, but there is one extremely practical aspect. Any attempts to assess parasitism of *M. pluviale* prior to pupation must include some measure of the *types* of larvae parasitized. Indeed, this stipulation also applies to assessments of larval diseases.

At this point it is worth noting that there is no ideal mixture of Type I and Type II larvae that would provide an all-purpose colony capable of functioning with equal efficiency in all environments or at all population densities. It is interesting that the new infestations of 1956 that were sparse enough to have only one colony per tree generally consisted of active colonies that constructed several tents and travelled over most of the crown during their larval period. Similarly, many trees that contained 20, 50, or even 70 tents supported only colonies that made compact tents and always fed close to them.

On the proper hosts, there is no doubt that less active colonies are much better suited for survival at moderate population densities, since they obtain food near home and do not mingle enough to spread disease among colonies. Nevertheless, since they often subsist on a minimal food supply even when they are not crowded, they suffer greatly from crowding that overlaps the limited feeding areas of adjacent colonies. Therefore, instead of speculating on the proportions of types required for an all-purpose colony, it is better to

recognize that the Type II component can easily become too large, so that previously advantageous habits are exaggerated to the point where they become detrimental. This was very evident in old infestations in 1956. In contrast, there was no evidence that any colonies contained too many Type I larvae, though mortality from this cause could have escaped notice during the early instars in which it would occur.

A Working Hypothesis and Some Required Observations

The observations in this paper provide material for a working hypothesis for further research on *M. pluviale*, but it will be necessary to follow local populations through at least one complete fluctuation in numbers before a completely satisfactory hypothesis can be developed. Meanwhile, the available evidence provides some guidance.

First, differences exist in the sensory physiology, rate of development, and survival ability of individuals that have far-reaching consequences in both individual and colonial life. Secondly, natural colonies contain different proportions of the different types of individuals and this affects both the course of their development and their chances for survival. Thirdly, increasing populations resident in one locality for several years show marked increases in numbers of sluggish colonies, whereas increasing populations introduced into new areas at first consist largely of active colonies. In fact, the farther they are from source areas, the more active colonies they contain, until the most distant new infestations consist entirely of active colonies that must contain high proportions of active and very small proportions of sluggish individuals. Therefore, since the 1956 outbreak showed this much variation within an aging population, it may be used as a reasonable model of what might happen through the years of any outbreak from its initial upsurge to its eventual decline. In other words, it is probable that the proportions of different types per colony vary from year to year throughout the course of a whole outbreak as well as among different infestation intensities within an outbreak, though it does not follow that they vary in the same manner.

The working hypothesis, therefore, may be reduced to these terms. A generation contains a basic range of variation that renders it more or less capable of surviving a given environmental pressure. Changing environmental pressure may move the range of this variation in one direction for successive years. Nevertheless, though annual changes in variability may be partly reflections of comparable changes in some environmental factors, the variability, in turn, may be capable of affecting the subsequent efficiency of some of these factors. Among other things, it may affect the manner in which the next generation is distributed and thus influence the course of local changes in population density. Stated in these terms, the hypothesis has elements in common with ideas expressed recently by Franz (2), Chitty (1), and Kennedy (7) and is, indeed, capable of the further generalizations discussed in the next section.

During this first season, it was impossible to obtain conclusive evidence concerning the origins of the different types of individuals. Possible genetic factors could not be evaluated as easily as some of the environmental factors, but some information on their relative importance should accumulate during the next few years. One point, however, is already clear. There may well be genetic roots, but weather, food quantity and quality, and the actions of the individuals themselves exert profound influences that require further evaluation wherever the whole problem leads.

For example, weather not only affected the course of development during 1956 but also seems to have been the most important barrier to establishment of new infestations prior to 1956. The Saanich Peninsula was chosen for this study because its orientation and topography are such that temperature, radiation, and precipitation gradients across it change with changing direction of approaching air masses, and it was clear that the average direction of approaching storms was subject to annual variations (15). Most of the new infestations in 1956 were established in areas too wet and cold in 1955 to permit larval development, and 1956 colonies developed there only because of the mild spring already noted. Therefore, continued observations along these lines are required for the duration of the investigation.

Similarly, observations on pupal and adult sizes in relation to infestation type indicated that reduction of food quantity and quality during the larval stage certainly exaggerates any innate sluggishness and may even lead to an increase in numbers of sluggish individuals in the next generation. Preliminary experiments have been inconclusive, but tests with large Type I larvae showed that starvation for 72 hours or more at 23°–24° C. produced behavior during orientation to light exactly similar to the behavior of Type II larvae (cf. Table II). Moreover, ligaturing these starved larvae (13, 16) or feeding them produced their original pattern of responses. To date, however, such drastic treatment has not permitted survival. Furthermore, it has been impossible to induce sluggish larvae to consume sufficient food to show any change in their responses. Nevertheless, this work suggests another approach to the problem of origins of types, and further observations seem warranted.

Sampling of field populations was kept to a minimum during 1956 because it was clear that indiscriminate sampling would only reproduce the difficulties encountered during analyses of the egg-mass data shown in Table I. With the present sounder bases, however, it should be possible to make annual assessments of the proportions of the different types emerging from egg masses collected with due attention to infestation history. Similarly, parasitism and disease surveys can be placed on a sounder basis.

Much of the present work suggests that colonies existing between outbreak periods should be relatively active, but it is unwise to speculate too much on their probable proportions. Until this work was done, any entomologist asked to describe tents of *M. pluviale* or *M. americanum* would have given a good description of a compact tent. Now we can recall elongate tents in previous outbreaks, but have no special recollection of such tents between

outbreaks. This may be a result of cursory observations, but it would be wise to withhold judgment. For example, no adults from the May 26 pupation by active larvae could be found in the field, and the fate of really sluggish colonies has already been noted. Residual populations undoubtedly will present a confused picture for a year or two, but the stabilized low population may prove to be more intermediate than anticipated. In 1956, the active colonies that appeared in new infestations were distributed at the low density of one per tree, but they were deposited by adults from near-peak populations, and there is no reason to suppose that present requirements for best survival at low density are the same as those for best survival during the future low point. The environment then will be different, and the few colonies that exist will be relatively isolated from one another. In sparse populations where individual colonies are really isolated, some reduction in wandering and some concentration of feeding and pupation near the tents may be advantageous if they reduce predation or accidents and increase mating opportunities. Consequently, detailed studies of the composition of natural colonies are required through the whole fluctuation in abundance.

General Applications

Kennedy (7) has pointed out that the environmental requirements and responses of locusts and other insects are not constant, but are subject to change within and between generations. Moreover, Franz (2) and Chitty (1) recently have suggested that adverse effects of changes in the nature of populations may be reflected in the vitality of their descendants. The western tent caterpillar, therefore, should not be treated as a special case just because its colonial life has proved more complicated than hitherto suspected. Instead, many of the analytical methods developed for its study could be applied quite directly in investigations of other animals. In fact, all that is necessary for further generalization of the concepts in the preceding section is replacement of the terms, "Types I and II", by more familiar terms to express the ranges of variability all organisms exhibit in their responses and requirements. The usual approach to population problems must be modified, however, before these ideas and methods can be properly applied to other animals.

Arguments concerning the relative merits of environmental factors associated with changes in population density have been worked and reworked with such care that many of them have lost some essential ingredients—the animals with which we are so concerned. We allow for individual variation in studies of their life cycles or their physiology, but tend to disregard it when we consider their changes in abundance. Then, we rely less on biological events than on mortality records, and tend to derive and test theories by contemplating these obituaries. Certainly, populations are not such inert masses as these records suggest, and lists of deaths do not indicate the qualities of the survivors. Indeed, even life tables, a promising route to more satisfactory theories when they are supported by intensive investigations (10, 11),

presently submerge most data on quality in their columns—notably in the one for unexplained mortality. Consequently, if we wish to study the role of individual differences in the population dynamics of an animal, we must revise field sampling systems to include data on the proportions of different kinds of individuals within and between generations.

Preliminary analysis to detect behavior differences or variability in responses to physical stimuli such as light, heat, and moisture seems to be the most promising approach where insects are concerned. Individual differences in efficiency of orientation are not confined to colonial forms, and differences in amount of activity are common. Therefore, some preliminary tests to determine what part of an observed difference remains unmodified by changes in test conditions should provide a rapid method for separating groups for further assessments of feeding habits, developmental rates, and survival abilities. A comparatively brief laboratory study of this nature is bound to reveal other differences in habits, size, or color marked enough to be incorporated in regular field sampling programs. Once this stage is reached, methods for assessing changing proportions within field populations are at hand.

These suggestions neither include unjustifiable assumptions nor minimize the difficulties involved in any search for characteristic differences. The assumptions are valid enough for inclusion in any working hypothesis, since they are based on the common observation that some differences in skill or total activity are simply reflections of deeper physiological differences among individuals. Consequently, the problem is to design skill or activity tests that will be adequate indicators of these underlying differences. Difficulties in designing suitable tests are not insuperable, and can be overcome by careful observation and consideration of the life of the animal in natural situations. Close attention to life history details will suggest experimental arrangements in highly artificial circumstances that will reveal valid and consistent differences capable of further exploitation.

For example, young larvae of the spruce budworm, *Choristoneura fumiferana* (Clem.), orientate very precisely to single lights (13), so that any attempt to classify them by differences in this response would be apt to fail. On the other hand, when they are properly acclimated they show different responses to moisture in gradient form (14), so that differences observed there might provide a starting point for further tests. The spruce budworm is solitary, but evaporation is important in its life, and recent work by Greenbank (4) suggests that its populations might prove separable by methods similar to some presented here.

The present methods can be extended almost intact to other tent caterpillars such as *M. americanum* and *M. disstria*, since the same kind of difference was observed among their first-instar larvae. Other colonial insects such as *Hyphantria textor* Harr. and *Halisidota argentata* Pack. exhibit individual differences in activity in dark–light chambers (16) and, in addition, show quantitative differences in the amount of time individuals spend in light in

these chambers at any constant temperature. Active larvae move often enough to cross boundaries frequently, whereas less active larvae do so less often. Consequently, differences between such individuals may be expressed in terms of percentage time in dark or light, number of boundary contacts, stops, or head movements per unit time, or rates of travel. Moreover, Long's studies (8, 9) on subsocial behavior in Lepidoptera and the effects of population density on their larvae strongly suggest that similar methods could be employed with the solitary and colonial insects he used. In addition, Ghent's work (3) on the initial establishment of young sawfly larvae suggests another method of separation of individuals on the basis of their first reaction when presented with food. Some may begin to chew unaided, whereas others cannot. Finally, it is clear that some tests of this nature may be simple enough to apply in the field. This can be done with western tent caterpillars and, with such insects, the investigator simply adds a battery of skill and activity tests to his other sampling methods.

Conclusions

1. When colonies of *M. pluviale* emerge from their egg masses, from 0–38% of the first-instar larvae are capable of directed travel in the absence of silk trails laid by other individuals. The remaining larvae are undirected except when they are on silk trails and, even there, they require frequent stimulation by the more active, directed larvae to keep them from clustering. In fact, some are so sluggish that they seldom move under any circumstances.

2. In light intensity gradients, the different types of larvae may be distinguished most reliably by the number of times they make lateral comparisons greater than 45° off their line of travel. Directed Type I larvae in their active periods make no more than three such movements per minute, and often make none. During their intermittent rest periods, or after starvation for 72 hours or more when they are nearly mature, they cannot be distinguished from the other larvae. The less directed Type II larvae make more than three such movements per minute—often as many as 15, and seldom less than six.

3. When the different types are separated into groups in the laboratory, only Type I larvae are capable of establishing themselves on food on twigs, but pure groups of active Type II larvae can become established on leaves in jars. Sluggish Type II larvae must be placed directly on their food if they are to survive. Mixing Type I and active and sluggish Type II larvae together permits establishment of active Type II larvae on twigs, and some sluggish larvae also may become established in such instances.

4. In individual rearings, Type I larvae regularly eat more and develop more rapidly than the others, averaging 3 days less than active Type II and 7 days less than sluggish Type II larvae between eclosion and pupation. Moreover, they generally pass through only five instars, whereas the others frequently have six. In groups in jars, these advantages may be destroyed by removing the small amounts of silk produced daily by small groups of Type I larvae or

by changing their food infrequently. On branches, however, where groups of larvae must construct tents and leave them to reach food just as in the field, Type I groups or mixed groups of Type I and active Type II larvae regain the advantage. Other kinds of larvae spend too much time clustered on their tents and do not feed often enough. Consequently, more active groups pass through four instars 7 days faster than pure Type II groups.

5. Experimental colonies of Type I, or Type I plus active Type II, larvae placed on branches in the laboratory or in the field construct more than two tents during their first four instars. They seldom suffer from widespread disease within their colonies because they change tents frequently and seldom rest long in clusters between feeding periods. Pure Type II colonies construct fewer tents and spend much time clustered upon them. Consequently, when disease occurs within their colonies it spreads quickly and generally kills all larvae.

The two kinds of colonies also construct different types of tents. Active colonies make elongate tents, whereas less active colonies make compact tents in which all sides are nearly equal.

6. Natural colonies in the 1956 outbreak in Saanich Peninsula, British Columbia, showed differences consistent with those exhibited by experimental colonies. The more active colonies constructed several elongate tents and fed over many meters of branches whenever weather was favorable. Consequently, some of them completed their larval stage 3 weeks or more ahead of less active colonies. Less active colonies tended to enlarge their original tent and to feed close to it, with the result that they often fed on previously damaged food. The most sluggish spent much time clustered even when weather was favorable, and many never pupated.

7. Surveys of different infestations showed that those newly established in 1956 contained a high proportion of colonies that made only elongate tents. In fact, only elongate tents occurred in new infestations more than 0.5 mi. from older infestations. In contrast, old, heavy infestations a mile or more in extent consisted largely of colonies that formed compact tents, and many trees with 20, 50, or 70 tents contained only the compact type.

8. Laboratory-reared adults show differences in spontaneous activity analogous to those exhibited by the larval types from which they came. In 1956, adults from field-collected pupae from the different types of infestation also showed similar activity differences, with high proportions of sluggish adults emerging in older infestations. Consequently, this evidence together with that from tent surveys indicates that more active adults are responsible for establishing new, distant infestations that consist initially of active colonies.

9. Consideration of laboratory results and the 1956 infestations suggests that the proportions of different types in a colony should vary not only between infestations of different ages in 1 year but also from year to year throughout the course of an outbreak. Because each type has certain disadvantages, however, it is unwise to speculate on the composition of the population at minimal density.

10. Results of other investigators indicate that the western tent caterpillar should not be considered to be a special case simply because it is a colonial insect. There is mounting evidence that the requirements and responses of animals are not constant within and between generations, and that changes in the nature of populations may be reflected in the vitality of their descendants. Therefore, the role of individual differences in the population dynamics of any animal is worth further consideration.

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THE GROSS AND MICROSCOPIC ANATOMY OF THE DIGESTIVE TRACT OF THE OYSTER CRASSOSTREA VIRGINICA (GMELIN)[1]

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Abstract

The gross and microscopic anatomy of the digestive tract of *Crassostrea virginica* (Gmelin), the common oyster of commerce of the North Atlantic Coast, is described. The dorsoventrally compressed mouth bounded by two pairs of labial palps leads into a crescentic oesophagus, thence to the anterior chamber of the stomach from which a complex caecum extends into anteriorly and posteriorly directed spiral appendices. The posterior chamber of the stomach bears a chondroid gastric shield and leads into an elongated chamber which is incompletely divided by two typhlosoles into a style-sac and mid-gut. The intestine is divisible into ascending, median, and descending limbs, the latter merging into the rectum which terminates on the dorsal surface of the adductor muscle. Extensively branched tubular digestive diverticula exit from the stomach by a series of ducts along the margin of the caecum and the posterior stomach. The complete digestive tract is lined by a simple columnar epithelium which is ciliated throughout with the exception of the upper lip or fused external palps, the lower side of the gastric shield in the posterior stomach, and the tubules of the digestive diverticula. Mucous secreting and eosinophilic epithelial cells occur in varying numbers along the course of the tract. Phagocytes are present between the lining epithelial cells, among the peripheral collagenous and muscle fibers, as well as in the lumen of the tract. The gastric shield is shown to be intimately attached to the underlying epithelium by a central clip as well as by minute cytoplasmic processes. The anatomical relationships are compared with various lamellibranchs including the Chilean oyster, *Ostrea chilensis* Philippi; the European oyster, *Ostrea edulis* L.; and the Portuguese oyster, *Gryphea angulata* Lamarck.

Introduction

Crassostrea virginica (Gmelin), the Atlantic coast oyster of commerce, occurs intermittently in coves, bays, and estuaries, and at the mouths of tidal rivers along the eastern seaboard of North America from the south shore of the Gulf of St. Lawrence to the Gulf of Mexico. Although Clark (1920 (6)) has made the statement that the oyster is the best known marine animal in the world, both physiological and pathological investigations have been seriously curtailed by the inadequacy of descriptive data relative to its gross and microscopic anatomy. Brooks (1880 (4)) in a brief account of the development of the oyster *Crassostrea* (*Ostrea*) *virginica* described the visceral mass as comprising a folded oesophagus, an irregular stomach surrounded by a dark greenish "liver", and a convoluted intestine. Ryder (1880 (26)) subsequently outlined the course of the digestive tract of the same species referring to the internal foldings of the stomach, the course of the intestine, and the extent of the "liver". Dahmen (1923 (7)) gave a comprehensive account of the gross structure of the tract of *Ostrea chilensis*

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Philippi and also referred to its histology. In an introduction to a physiological study of *Ostrea edulis* L., Yonge (1926 (33)) described the gross anatomical relationships of the tract briefly and the microscopic anatomy in somewhat more detail. Leenhardt (1926 (17)) dealt largely with the histological structure of the tract of the Portuguese oyster, *Gryphea angulata* Lamarck (*Crassostrea angulata* Gunter, (1950 (14)), and made only minor references to gross anatomical relationships. The present study of the digestive tract of *Crassostrea virginica* was accordingly undertaken because of the paucity of data on both the gross and microscopic anatomy of the oyster and of this species in particular.

Materials and Methods

All oysters used in this investigation were obtained from Malpeque Bay, Prince Edward Island, Canada. Initial dissections were carried out on fresh specimens as well as following preservation and hardening in 5% formalin. The gross anatomical relationships of the lumen of the tract were determined from casts made by injecting alkaline latex or vinylite resin through the mouth and the anus of fresh specimens and subsequently removing the surrounding tissues.

For histological studies, the relative merits of a number of the more common fixatives were determined and, of these, Davidson's solution of the following formula proved most efficacious:

formalin (analytical reagent 35%)	20 parts
glycerin	10 parts
alcohol, 95%	30 parts
glacial acetic acid	10 parts
water (sea water where available)	30 parts

After fixation for 24 hours, specimens were stored in a solution of the same formula minus the glacial acetic acid. Following dehydration, toluol was employed for clearing and Tissuemat of melting point 60° to 62° C. for imbedding. Sections were cut at 5 to 10 μ and stained with Erhlich's haematoxylin and Triosin (Galigher, 1934 (12)), iron haematoxylin, Mallory's triple stain, Van Gieson's picrofuchsin, and cresyl echt violet.

Newly-set spat from less than 1 mm. to 10 mm. in length were fixed in Bouin's fluid and cleared in creosote or clove oil with or without prior staining in alum-cochineal. Serial sections cut at 10 μ were counterstained with light green.

Gross Anatomy of the Digestive Tract

Terminology

The terminology of Pelseneer (Nelson, 1938 (23)) with regard to the axes of symmetry of the oyster will be followed in this paper. Accordingly the principal axis of the oyster lies in a line drawn through the mouth and the anus. The hinge is anterodorsal, the palps and forward portions of the gills project ventrad, while the hinder portions of the gills extend both ventrad and posteriorly.

General Anatomical Relationships

The right valve of *Crassostrea virginica* is flattened and usually uppermost in naturally-setting spat, while the left valve is cupped toward the hinge to accommodate the visceral mass. Removal of the right valve exposes the right lobe of the mantle, whose thickened and darkly pigmented free margin bears minute tentacles. The single adductor muscle is located slightly posterior to a line bisecting the principal axis and somewhat nearer the dorsal than the ventral margins of the valves. The crescentic posterodorsal division of the muscle is composed of smooth fibres, while the anterior "catch" division consists of striated fibers (Yonge, 1926 (33)). On the removal of the right lobe of the mantle (Fig. 1), the visceral mass, the gills, and the pericardial cavity are exposed. The visceral mass is ovoid anteriorly and forks posteriorly. The ventral branch is triangular with its apex extending to the mid-level of the adductor muscle. It comprises a posterior diverticulum of the stomach, the so-called style-sac, fused lengthwise with the first segment of the intestine or the mid-gut, as well as the ascending or recurrent limb of the intestine. The cylindrical dorsal branch of the visceral mass encloses the rectum, which terminates on the dorsal margin of the adductor muscle. The ventral anterior margin of the visceral mass is surmounted by two pairs of labial palps whose posteriorly-directed free ends overlap the anterior margins of the gill plates.

Labial Palps and Mouth

The labial palps are roughly triangular plates with an acute apex directed posteriorly (Figs. 1 and 2). Their ventral margins are somewhat crescentic while the dorsal margins are straight and fused anteriorly to the ventral surface of the visceral mass. The outer pair are slightly larger than the inner pair and, except at the anterior roots, are entirely free from the inner palps. The lateral surfaces of the external palps and the medial surfaces of the internal palps are smooth or plane. The adjacent medial surfaces of the external palps and the lateral surfaces of the internal palps are coarsely fluted with ridges directed obliquely towards the mouth opening. The latter takes the form of a dorsoventrally compressed slit at the outer margin of the valve-hinge extremity of the body. Ryder (1880 (26)) described it as appearing between the upper median angles of the palps. The outer pair of palps extend anteriorly and dorsally around the mouth where they fuse in the form of a hood or upper lip. The inner palps are united with each other along their mid-dorsal line for one-half or more of their length and their fused anterior ends are elevated as a fleshy ridge or lower lip. Dahmen (1923 (7)) and Yonge (1926 (33)) have also shown, for *O. chilensis* and *O. edulis* respectively, that unlike the majority of lamellibranchs, these species have the inner and outer palps of the two sides fused in the region of the mouth. In addition, however, in the latter species the outer palps are united for one-fourth of their length so that the mouth is entirely enclosed.

Mouth Cavity and the Oesophagus

The mouth opening leads into an elongated dorsoventrally flattened mouth cavity (Fig. 19). Ryder (1880 (26)) noted that "the mouth is so wide the animal can scarcely be said to have an oesophagus". Leenhardt (1926 (17)) described an elongated passageway between the oral opening and the anterior oesophageal orifice in *G. angulata* and suggested its analogy to a pharynx. The presence of this passageway would appear to be a characteristic of the genus *Crassostrea*, since in *O. chilensis* (Dahmen, 1923 (7)) and *O. edulis* (Yonge, 1926 (33)) the mouth leads directly into a short oesophagus. In *C. virginica* the oesophagus arches posterodorsally at an angle of approximately 45 degrees to the long axis of the palps. It measures approximately one-fifth of the maximum anterior–posterior length of the visceral mass and enters the anterior chamber of the stomach at the junction of the latter with the caecum (Figs. 3, 4, 5).

Stomach

The stomach (Figs. 3, 4, 5, 21, 22) is a saccular organ centrally located in the posterior two-thirds of the ovoid portion of the visceral mass. It is completely surrounded by the greenish-brown tubules of the digestive diverticula. The stomach proper is divisible into a smaller anterior chamber grading into a somewhat larger posterior chamber. The anterior chamber appears as an enlargement at the base of the oesophagus. An extensive complex outpouching or caecum arises from its left ventral surface, and takes the form of an oblique channel, with spirally directed anterior and posterior limbs or appendices. The anterior appendix is smaller than the posterior appendix and projects dorsad along the base of the oesophagus (Fig. 4). It comprises one and one-quarter to one and one-half turns which follow a counterclockwise direction. The large posterior appendix appears bandlike

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FIG. 1. Gross anatomical relationships of *C. virginica*, as viewed with the right valve and the right mantle removed. The visceral mass is heavily outlined. A.M., adductor muscle; G.P., gill plates; L.MA., left mantle; L.P., left labial palps; L.V., left valve; P.C., pericardial cavity; V.M., visceral mass.

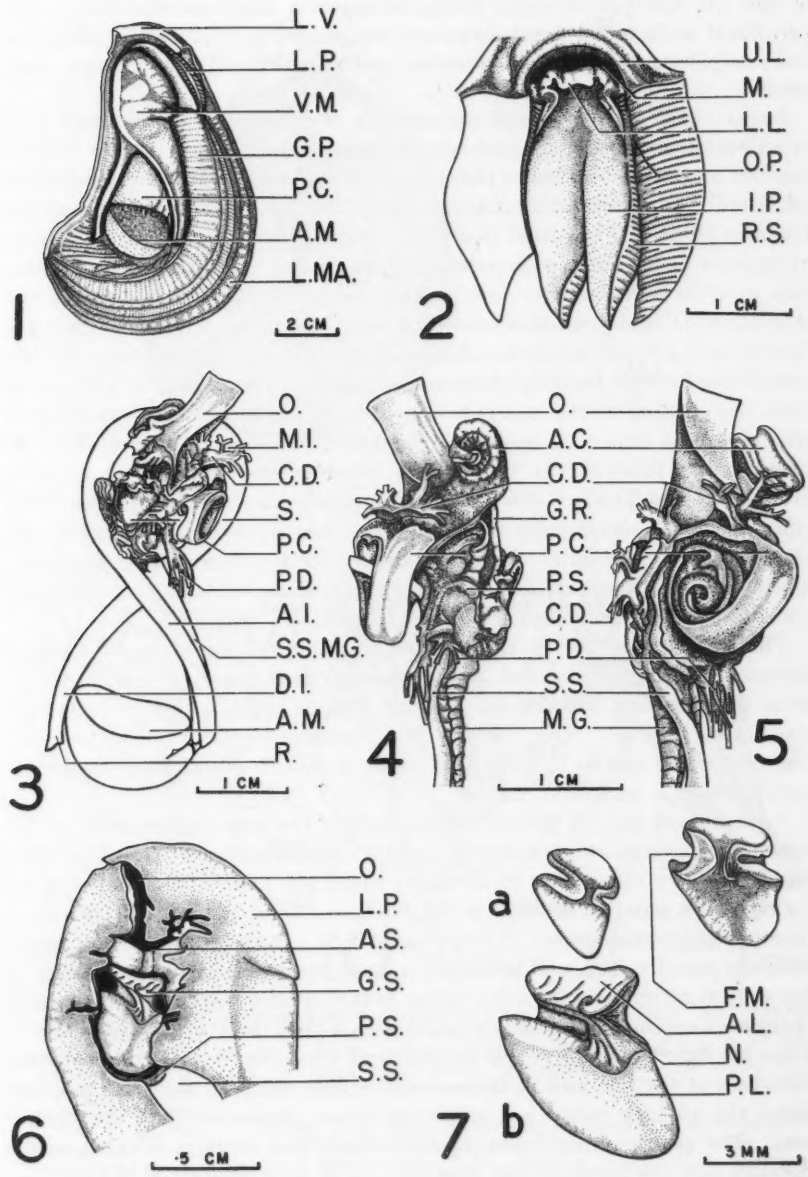
FIG. 2. Mouth opening from the anteroventral aspect, with the inner and outer labial palps spread laterally. I.P., inner labial palp (left); L.L., lower lip; M., mouth; O.P., outer labial palp (left); R.S., ridged surfaces; U.L., upper lip.

FIG. 3. Latex mold of the lumen of the digestive tract, exclusive of the tubules of the digestive diverticula, viewed from the right lateral aspect. A.I., ascending limb intestine; A.M., adductor muscle; C.D., caecal ducts of digestive diverticula; D.I., descending limb intestine; M.I., median limb intestine; O., oesophagus; P.C., pericardial cavity; P.D., posterior gastric ducts of digestive diverticula; R., rectum; S., stomach; S.S.M.G., style-sac and mid-gut.

FIGS. 4 and 5. Latex mold of the lumina of the oesophagus and stomach viewed from the left lateral aspect (Fig. 4) and the left ventral aspect (Fig. 5). A.C., anterior appendix of caecum; C.D., caecal ducts of digestive diverticula; G.R., gastric ridges for attachment gastric shield; M.G., mid-gut; O., oesophagus; P.C., posterior appendix of caecum; P.D., posterior gastric ducts of digestive diverticula; P.S., posterior chamber of stomach; S.S., style-sac.

FIG. 6. Dissection of the stomach with the right wall removed to show the internal ridges and gastric shield in situ. A.S., anterior chamber of stomach; G.S., gastric shield; L.P., labial palps; O., oesophagus; P.S., posterior chamber of stomach; S.S., style-sac.

FIG. 7. Typical gastric shields; (*a*) left lateral aspects, (*b*) medial aspect. A.L., anterior lobe; F.M., flexed margins forming clip; N., neck; P.L., posterior lobe.


L.V.
L.P.
V.M.
G.P.
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R.S.
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1 CM
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P.D.
A.I.
S.S. M.G.
D.I.
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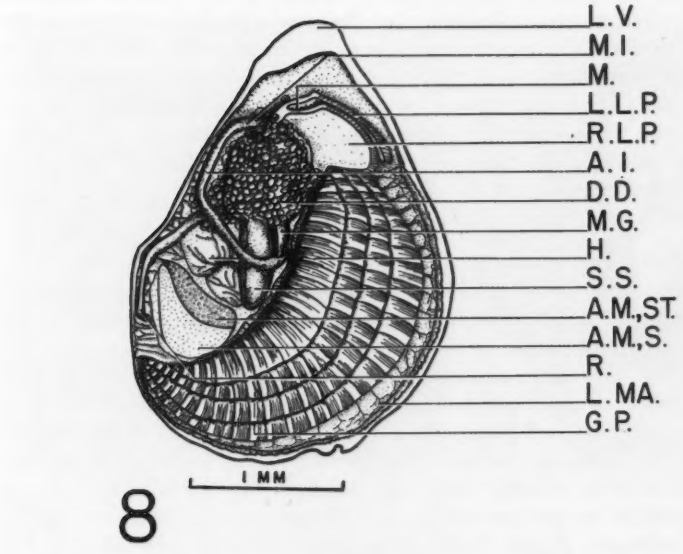
3
1 CM
O.
A.C.
C.D.
G.R.
P.C.
P.S.
C.D.
P.D.
S.S.
M.G.
4
1 CM
5
O.
L.P.
A.S.
G.S.
P.S.
S.S.
6
.5 CM
a
F.M.
A.L.
N.
P.L.
7
b
3 MM

and is directed ventrally and toward the right of the stomach. It consists of one and one-quarter turns which extend in a clockwise direction. The peripheral walls of the appendices are invaginated as typhlosoles, while the walls adjoining the anterior stomach are flattened and approximate each other.

Ryder (1880 (26)) described the stomach of *C. virginica* as an organ with prominent transversely directed internal folds, two of which lie in a ventral position and take the form of inward projecting lobes "which are themselves lobulated", and presumably correspond to the caecum and its appendices. Dahmen (1923 (7)) indicated that the stomach of *O. chilensis* is divisible into an anterior dorsal and a wide posterior chamber, the left side of which extends into anterior and posterior blind sacs. Yonge (1926 (33)) illustrated the stomach of *O. edulis* as a short compact organ, the internal walls of which are thrown into a series of ridges. A food-sorting caecum extends from the left posteriorly beneath the floor of the stomach and is connected by a deep groove with the opening of the mid-gut. Such a caecum is a much less complex structure than that of *C. virginica*. Leenhardt (1926 (17)) in describing *G. angulata* has followed the terminology of other authors such as Sabatier (1887 (27)) and Thiele (1886 (29)) for lamellibranchs, where the term "utricular stomach" corresponds to the anterior and posterior chambers of the stomach of *C. virginica*. He refers briefly to a "diverticulum stomach" which arises on the anterior, ventral surface of the utricular stomach and which probably is homologous with the caecum of *C. virginica*.

The internal surface of the anterior chamber of the stomach exhibits considerable irregularity and possesses numerous ridges. It is separated from the posterior chamber by a broad fold or ridge, which projects into the lumen (Fig. 6). This ridge is most prominent along the mid-ventral wall and would appear to serve as a means of directing food particles posteriorly through a narrowed channel.

An extensive area of the left ventral wall of the posterior chamber of the stomach is covered by a translucent gastric shield (Figs. 6 and 7). The latter consists of two main lobes or divisions which are joined by a narrow neck region. The anterior division is elliptical in outline and its shorter axis is directed anteroposteriorly. The posterior lobe is two to three times larger than the anterior lobe, and is roughly a quadrant with one radius serving as the ventral margin and the other radius as the anterior margin. The margins of the neck region, and the adjoining margins of both the anterior and posterior lobes are flexed, forming a clip arrangement which fits onto a corresponding elevation of the left wall of the stomach. The marginal flection extending along the anterior radius is considerably more prominent than the ventral one. The gastric shield bears a longitudinal crest or ridge which projects dorsally into the lumen of the stomach. It is most evident in the anterior lobe and becomes progressively reduced in the posterior lobe. The anterior lobe in addition bears a number of irregular prominences or teeth.



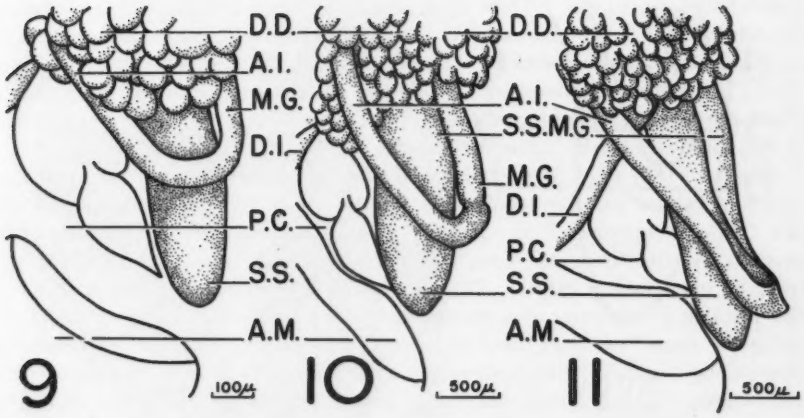


FIG. 8. Camera lucida drawing of cleared specimen of 3 mm. oyster spat viewed from right lateral aspect. A.I., ascending limb intestine; A.M.,S., adductor muscle, smooth; A.M.,ST., adductor muscle, striated; D.D., digestive diverticula; G.P., gill plates; H., heart; L.L.P., left labial palps; L.MA., left mantle; L.V., left valve; M., mouth; M.G., mid-gut; M.I., median limb intestine; R., rectum; R.L.P., right labial palp; S.S., style-sac.

FIGS. 9, 10, and 11. Camera lucida drawings showing relationship of style-sac and mid-gut of 3 mm., 7 mm., and 9 mm. oyster spat respectively, viewed from the right lateral aspect. A.I., ascending limb intestine; A.M., adductor muscle; D.D. digestive diverticula; D.I., descending limb intestine; M.G., mid-gut; P.C., pericardial cavity; S.S., style-sac; S.S.M.G., style-sac and mid-gut.

Nelson (1918 (22)) first coined the term "gastric shield" for the platelike structure of cartilaginous consistency at the point of contact between the anterior end of the style and the epithelium of the posterior stomach. For *C. virginica* he described it as a trilobular structure with the smallest of the three lobes concave on one surface forming a bowl-like depression possibly corresponding to the gastric clip arrangement as described here. The posterior lobe of the shield in the Malpeque Bay specimens is undivided, or marked only by a slight indentation of the posterior margin similar to that illustrated by Yonge (1926 (33)) for *O. edulis*.

Style-sac and Mid-gut

Just caudad to the gastric shield the posterior stomach leads into an elongated outpouching (Fig. 2), which occupies most of the ventral arm of the visceral mass. At the anterior end, two lateral valvular folds project into the lumen of the outpouching and continue posteriorly as the smaller or right and the larger or left typhlosoles, which incompletely divide the passageway into two parallel channels (Fig. 25). The mid-gut comprises the ventral channel and the style-sac lies somewhat dorsal to it. In cross section the lumen of the style-sac usually appears larger than that of the mid-gut, and irregularly circular to oval in outline in contrast to the somewhat laterally compressed mid-gut. Both typhlosoles show considerable variation, but the right one usually appears as a relatively low broad fold, while the left is somewhat more conical. These typhlosoles correspond to those of *O. chilensis* (Dahmen, 1923 (7)) and *O. edulis* (Yonge, 1926 (33)), but are somewhat less prominent than those of other lamellibranchs including *Anodonta* (Nelson, 1918 (22)), *Mya* (Edmondson, 1920 (8)), and *Ensis* (Graham, 1930 (13)). The lumina of the style-sac and the mid-gut enter a common posterior chamber at the mid-level of the adductor muscle.

Sabatier (1887 (27)) in describing *Mytilus edulis* first termed the style-sac and mid-gut a "tubular stomach". Purdie (1887 (25)) demonstrated that the "pyloric appendix" in *Mytilus latus* comprised the crystalline-style caecum and the direct intestine, which were partially separated by two overhanging longitudinal ridges. Dahmen (1923 (7)) referred to this portion of the tract in *O. chilensis* as a stomach–intestine while Leenhardt (1926 (17)) using the term "estomac tubulaire" for *G. angulata* stated that it included a caecum or "canal cylindro-conique" separated from a parallel channel by two ridges. Nelson (1918 (22)) also described this region of the digestive tract for a number of lamellibranchs, including *C. virginica*, as consisting of two tubes incompletely separated by typhlosoles, and Yonge (1926 (33)) corroborated this for *O. edulis*.

The rodlike crystalline style is enclosed in the groove of the style-sac and extends forward to the surface of the gastric shield in the posterior chamber of the stomach. It is composed of gelatinous layers enclosing a central fluid (Fig. 22). The style was first described in detail for *C. virginica* by Nelson (1918 (22)) and does not appear to differ from that of *O. chilensis* (Dahmen, 1923 (7)), *O. edulis* (Yonge, 1926 (33)), or *G. angulata* (Leenhardt, 1926 (17).

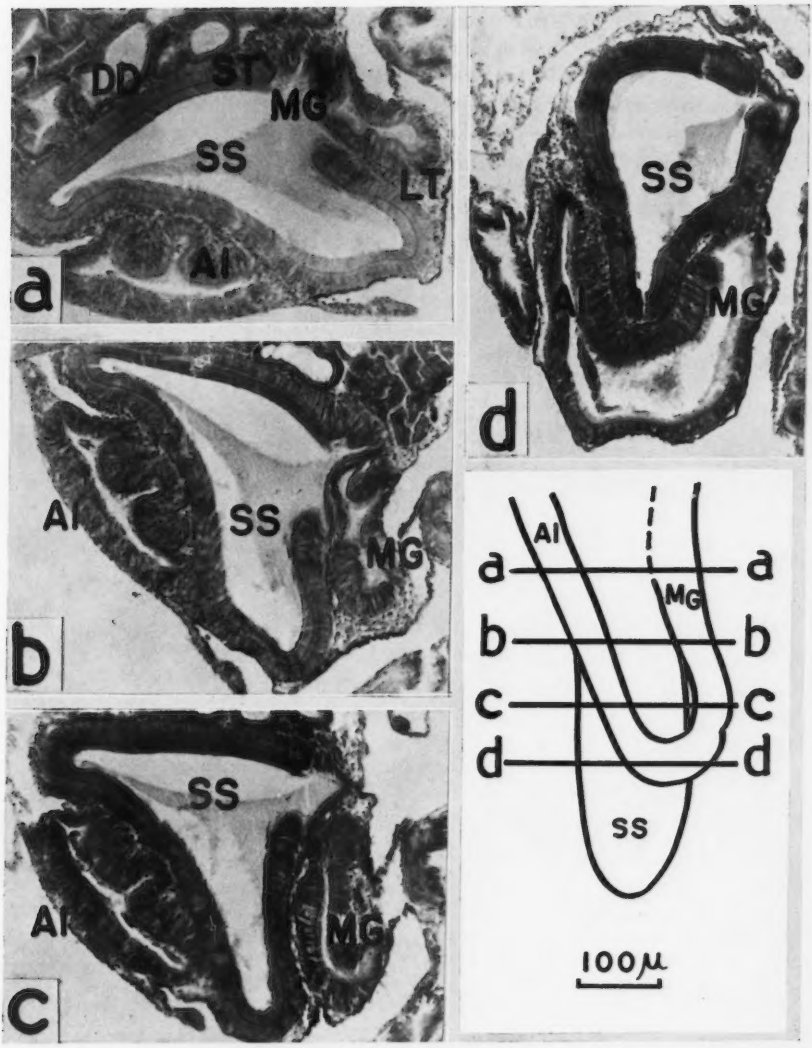


FIG. 12. Photomicrographs of cross sections of style-sac, mid-gut, and ascending limb of intestine of a 5 mm. oyster spat at levels as indicated by horizontal lines on diagrammatic outline. Alum cochineal and light green, 7 μ.

(*a*) Fused style-sac and mid-gut.

(*b*) Adjacent walls of style-sac and mid-gut degenerating prior to fusion.

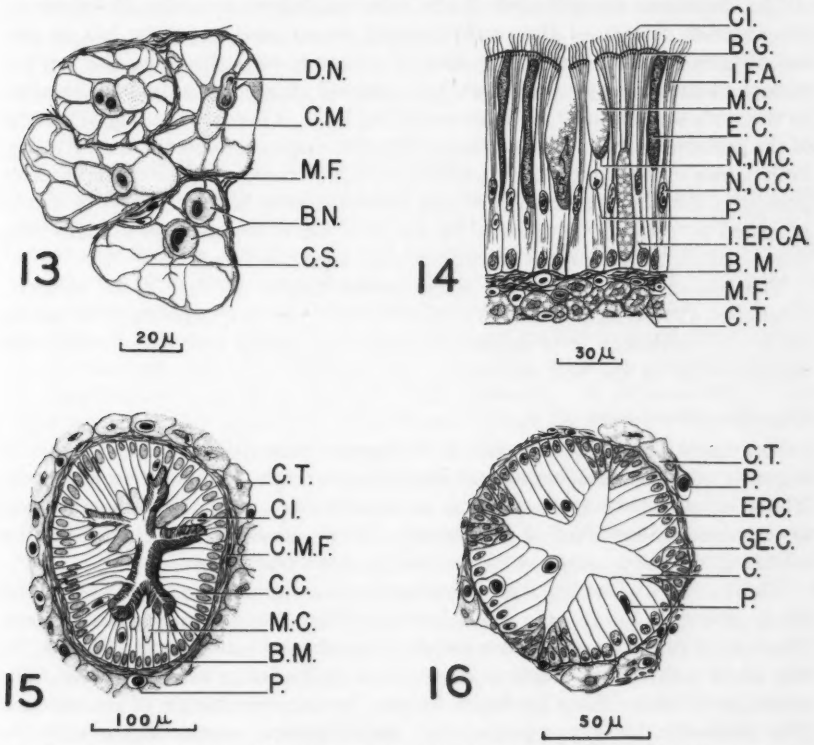
(*c*) Style-sac and mid-gut discreet, ventral wall of style-sac grooved and non-ciliated.

(*d*) Style-sac with mid-gut looping over right surface.

A.I., ascending limb intestine; D.D., digestive diverticula; L.T., larger typhlosole (left); M.G., mid-gut; S.S., style-sac; S.T., smaller typhlosole (right).

Intestine—Ascending, Median, and Descending Limbs and Rectum

From the common chamber at the posterior end of the style-sac and mid-gut the intestine can conveniently be divided into ascending or recurrent, median or vertical, and descending limbs, and the rectum. The ascending limb passes anteriorly by an abrupt flexure from its origin at the common posterior chamber of the style-sac and mid-gut (Fig. 3). It exhibits many variations in spatial relationship with reference to the style-sac and mid-gut. Most frequently it



Camera lucida drawings (semidiagrammatic) of histological sections of the digestive tract of *Crassostrea virginica*

FIG. 13. Typical Leydig cells or Langer's vesicles. B.N., binucleate cell; C.M., cytoplasmic mass; C.S., cytoplasmic strands; D.N., dividing nucleus; M.F., muscle fibers.

FIG. 14. Characteristic ciliated epithelium from style-sac. B.G., basal granule; B.M., basement membrane; CI., cilia; C.T., connective tissue (collagenous fibers and Leydig cells); E.C., eosinophile; I.EP.CA., intraepithelial canal; I.F.A., intrafibrillar apparatus; M.C., mucous cell; M.F., smooth muscle fibers; N., C.C., nucleus of ciliated epithelial cell; N., M.C., nucleus of mucous cell; P., phagocyte.

FIG. 15. Transverse section of typical large duct of digestive diverticula. B.M., basement membrane; C.C., ciliated epithelial cell with heavy cuticular border (Cell Type I); CI., cilia; C.M.F., circular muscle fibers; C.T., connective tissue (collagenous fibers and Leydig cells); M.C., mucous cell; P., phagocyte.

FIG. 16. Transverse section of typical tubule of digestive diverticula. C., crypt; C.T., connective tissue; EP.C., secretory (and absorptive) epithelial cell; GE.C., generative cell; P., phagocyte.

passes forward on the right ventral surface of the latter for some distance before flexing obliquely anterior to the pericardial cavity. Less frequently the flexure is more abrupt and it is parallel to the dorsal surface of the style-sac and mid-gut, and sometimes it forms the dorsal limb of a large U with the style-sac and mid-gut as the ventral limb. It subsequently follows the curvature of the dorsal surface of the ovoid visceral mass lying somewhat peripheral to the digestive diverticula.

The median or vertical limb of the intestine follows a crescentic course on the anterior surface of the ovoid visceral mass, passing to the left of the oesophagus. As the descending limb it curves posteriorly along the ventral surface of the ovoid visceral mass, and crossing obliquely to the left, anterior to the style-sac, mid-gut, and the ascending limb, it borders the dorsal margin of the pericardial sac. Aside from a slight decrease in diameter, the descending limb passes without gross demarcation into the rectum. The latter terminates dorsal to the smooth division of the adductor muscle to which it is firmly attached by connective tissue. Its posterior tip is mounted on a connective tissue papilla although the immediate anal region is free.

The course of the intestine is essentially similar to that of *O. chilensis* (Dahmen, 1930 (7)), *O. edulis* (Yonge, 1926 (33)), and *G. angulata* (Leenhardt, 1926 (17)) although it is apparently relatively longer and with less abrupt flexures than in the last species.

Digestive Diverticula

The digestive diverticula consist of irregular brownish-green lobules, which together with the oesophagus and stomach constitute the ovoid visceral mass. These lobules, bound together by an interlobular connective tissue stroma, are exposed on removal of the mantle. They consist of ducts and tubules which fill all the interspaces about the digestive tract proper.

The ducts by which the digestive diverticula communicate with the stomach occur in two main groups: the caecal ducts and the posterior gastric ducts. The caecal ducts may be conveniently divided into four groups (Figs. 4, 5), the most anterior of which represents the fusion of two large ducts. The apertures of these ducts are located along the anterior margin of the caecum. The posterior ducts, comprising two major groups, communicate with the posterior chamber of the stomach on the left side. Dahmen (1923 (7)) described six major ducts in *O. chilensis*, four of which open along the caecum, the remainder into the right and left sides of the stomach proper. Yonge (1926 (33)) indicated the presence of two large ducts opening on each side of the stomach in *O. edulis*, and Leenhardt (1926 (17)) found that several canals open into the "utriculaire" stomach in *G. angulata*.

Typhlosoles of the Intestine

At the posterior termination of the style-sac and mid-gut channels, the larger (left) and the smaller (right) typhlosoles (Fig. 25) unite into a single typhlosole with a median groove. This typhlosole, which comprises about one-half of the wall of the gut, continues throughout the remainder of the

digestive tract to within a short distance of the anus. In the ascending limb it is deeply grooved and occupies the left wall. With the flection of the intestine into the median limb it forms the anterior wall, while in the descending limb and rectum it comprises the right wall.

The external contours of the intestine and the surface of the typhlosole appear smooth and rounded when large quantities of food are present in the lumen. If there is little or no food or mucous, characteristic constrictions and dilatations in the opposite wall of the intestine are impinged on the typhlosole. These are evident throughout the entire tract with the exception of the anterior portion of the ascending limb and the median limb, both of which are somewhat deeply imbedded in the dense mass of the digestive diverticula.

Development of the Tract Following Setting

Development of the digestive tract in the larva of *C. Virginica* has been described by Brooks (1880 (4)), Stafford (1913 (28)), and Chestnut (1949 (5)). These descriptions, however, do not include accounts of the metamorphosis of the tract from the spat to the adult condition. The stomach is evident in early larval stages as an anterior enlargement of the semicircular tract. At the time of "setting" the style-sac is a blind posteriorly directed diverticulum of the stomach (Fig. 8). The mid-gut originates from the stomach slightly to the right and ventral to the base of the style-sac. It forms a loop on the right surface of the latter sac. The dorsal limb of the loop traverses the left surface of the stomach as the anlage of the ascending limb of the intestine. At the level of the base of the oesophagus it curves posteriorly, as the descending limb of the intestine and the rectum, to terminate on the dorsal surface of the adductor muscle. Subsequently the ventral wall of the style-sac and the closely approximating dorsal wall of the mid-gut become fused to one another (Figs. 9–12). This line of fusion is marked in the style-sac by a longitudinal groove of low sparsely ciliated epithelial cells. An initial thinning of the adjacent walls of both the style-sac and mid-gut is accompanied by a degeneration of the cilia and finally of the epithelial cells themselves, until only the basement membranes remain to be ruptured. This rupturing begins anteriorly and proceeds posteriorly. The smaller and larger typhlosoles extending the length of the style-sac and mid-gut are thus indicative of the right and left fused margins of the two channels. Fusion begins when the spat are between 3 and 5 mm. in length and appears to be complete in the majority of 10 mm. spat. The style-sac and mid-gut are thus a combined gastric and intestinal channel, as shown for *O. chilensis* (Dahmen, 1923 (7)) and for *O. edulis* by Yonge (1926 (33)) and Erdmann (1935 (10)), rather than simply a tubular stomach as Leenhardt (1926 (17)) has indicated in *G. angulata*.

Microscopic Anatomy of the Digestive Tract

The viscera of the oyster are bound together and supported by connective tissue consisting of Leydig cells alternately known as Langer's vesicles (Fig. 13), and delicate collagenous fibers. Leydig cells have been described for a

number of the molluscs (Bronn, 1935 (3)). In *C. virginica* (Fig. 13) they are multiangular and usually slightly elongated. Each cell possesses one or more vesicular nuclei which lie toward the periphery of a centrally-located mass of finely granular cytoplasm. Delicate cytoplasmic strands radiate from the central mass to the margin of the cell where they unite to form a thin border adjacent to the cell membrane. These cells are arranged at random except around blood vessels where the long axes radiate from the encircling collagenous fibers; and in the subepithelial and collagenous layers of the digestive tract and of the mantle. In the latter they form a circular layer with the long axes parallel to the lumen of the tract and the margin of the mantle respectively. Dahmen (1923 (7)) found the interspaces between the cytoplasmic strands to contain a clear fluid or very finely granular material. Bargeton (1941 (1)) indicated their importance in the storage of glycogen. In *C. virginica* these cells exhibit a seasonal variation in size. In early summer prior to spawning, they appear flattened, presumably owing to glycogen depletion and the pressure of the enlarging and permeating gonad. There is no marked change in the long axes, but the short axes are now reduced to from 20 to 40 μ. In late summer sometime after spawning, their long axes measure from 40 to 70 μ and their shorter axes from 35 to 60 μ.

The simple columnar epithelial lining layer of the digestive tract (Fig. 14) rests upon a homogenous basement membrane and is separated from the Leydig cell stroma by a somewhat dense, circularly arranged layer of delicate collagenous fibers. A few smooth muscle fibers, circularly and occasionally longitudinally disposed, usually permeate the periphery of the collagenous layer and partially or completely separate it from the underlying Leydig cells. The muscle fibres are sometimes fusiform, but occasionally appear to have blunt ends, a condition which may be partially attributed to the plane of section. The epithelium is ciliated throughout with the exception of the upper lip (fused external palps) where cilia are only sporadically present, the tubules of the digestive diverticula, and the lower side of the gastric shield in the posterior chamber of the stomach. The height of the epithelium varies from one region to another, as does the nature of the cilia. The cytoplasm is finely granular and the nuclei are large, oval in shape, and usually located from the middle to the base of the cell. The chromatin is sparse and tends to collect in coarse granules. One or more nucleoli are present near the nuclear membrane. An internal fibrillar apparatus appears to arise near the basement membrane in the form of elongated rods or fine fibrils. These fibrils converge as they pass toward the nucleus and thence spread in the form of a fan to merge into the basal granules near the free surface before penetrating the cuticle. There is considerable variation in the ease with which the internal fibrillar apparatus can be differentiated in different parts of the tract. Gutheil (1911 (15), 1912 (16)) described this apparatus in *Anodonta cellensis*, but did not observe it basal to the nucleus although Ellermann (1899 (9)) suggested that the fibrils extended toward the bases of the epithelial cells in the snail *Helix pomatia*. Mackintosh (1925 (20))

has indicated a similar internal fibrillar apparatus in *Crepidula fornicata* as has Yonge (1926 (33)) for *O. edulis*, and Leenhardt (1926 (17)) for *G. angulata*. A series of "intra-epithelial" canals in the basal third of the cells are similar to those demonstrated by Mackintosh (1925 (20)) and Yonge (1926 (33)). It is suggested that these canals lend tensile strength to the epithelium since they are filled with a densely-staining stringy or albuminous substance and penetrate the bases of the cells.

Secretory epithelial cells of the goblet type are interspersed amongst the ciliated cells. Their proportions and numbers vary from one region of the tract to another (Fig. 14). Some of these are actively secreting mucous cells, while others are apparently regenerating cells. Coarsely vesicular mucous granules or droplets are grouped as ovoid to spindle-shaped masses which extend variable distances from the free border toward the bases of the cells. The granules stain weakly with basic dyes although in early stages before taking on a vesicular character, they stain more intensely. The densely-staining nucleus is oval or crescentic in shape depending upon the volume of secretion. It is located in a position somewhat eccentric to the basal margin of the ovoid mass of secretion. The cytoplasm is finely granular and similar to that of the ciliated cells. Leenhardt (1926 (17)) first described the so-called eosinophilic cell in *G. angulata*. It is an elongated cell, laden with coarse granules, which may extend throughout most of the length of the cell, and cause it to be distended to a greater or lesser extent especially toward the free margin. Cells of this type can be recognized in the digestive epithelium of *C. virginica*. The granules can be stained with both iron and acid hematoxylin and also intensely with acid dyes, such as eosin. The densely-staining nucleus is spherical or oval in shape and located in finely granular cytoplasm toward the base of the cell. Nelson (1955 (24)) has suggested that these may represent a secretory phase of the mucous cell. Validity is loaned to this hypothesis in that there is sometimes considerable difficulty in distinguishing these cells from mucous cells, and wherever mucous cells are abundant, eosinophiles are sparsely represented. Dahmen (1923 (7)) and Yonge (1926 (33)) for *O. chilensis* and *O. edulis* respectively refer to only one type of secretory cell, the "mucous" gland, which occurs in various regions of the digestive tract. The nuclei appear to be identical with those of the ciliated cells. They fail to distinguish any cells which correspond to the eosinophiles of *G. angulata* (Leenhardt, 1926 (17)).

Wandering phagocytic cells from 7 to 10 μ in diameter occur in variable numbers in the lumen, among the epithelial cells and in the surrounding connective tissues of the entire digestive tract. Their small spherical nuclei are 4 to 5 μ in diameter and the chromatin occurs as fine densely-staining granules. The cytoplasm stains faintly with acid dyes. In fixed preparations they frequently exhibit numerous blunt pseudopodia. Some investigators refer to these cells as amoeboid cells or amoebocytes (Yonge, 1926 (33)), while others term them leucocytes (Mackin, 1951 (19)). Gutheil (1912 (16)) showed for *Anodonta* and Yonge (1923 (31)) for *Mya* sp. that large particles

of food can be phagocytized by them and hence they may play an important role in assimilation.

Table I gives a résumé of the specific characteristics of the lining epithelium and extraepithelial tunics of the digestive tract of *C. virginica*. The following descriptions will accordingly deal largely with certain distinguishing features of the various regions.

Labial Palps (Figs. 17, 18)

Each fleshy labial palp is composed of a core of connective tissue surmounted by simple columnar epithelium resting on a homogeneous basement membrane. The connective tissue consists of Leydig cells with some collagenous fibers, the latter, along with longitudinally arranged smooth muscle fibers, being largely concentrated as a layer beneath the basement membrane. The smooth surfaces of the palps, i.e. the lateral faces of the external palps and the medial faces of the internal palps, are bordered by a sparsely ciliated low columnar epithelium with or without a thin cuticle. Both mucous and eosinophilic cells may be interspersed amongst the ciliated cells. The adjacent surfaces of both pairs of palps bear prominent transverse ridges or flutings separated by grooves, and are covered by a heavily ciliated tall columnar epithelium with a distinct cuticle. In longitudinal sections the ridges present an oblique appearance, the proximal walls usually being straight, while the distal walls are characterized by shallow grooves, as Leenhardt (1926 (17)) illustrated for *G. angulata*. Mucous cells tend to be more concentrated at the bases of the furrows and become progressively reduced in numbers toward the summits of the folds. Yonge (1926 (33)) has shown in *O. edulis*, however, that unicellular mucous glands are almost exclusively confined to the vicinity of the summits of the folds. Phagocytes between the epithelial cells and longitudinally arranged muscle fibers under the epithelium of the furrows are also present in *O. edulis*.

Mouth Cavity and Oesophagus (Figs. 19, 20, 30)

In the region of the upper lip formed by the fused external palps the epithelium is low columnar and non-ciliated or irregularly ciliated. The free surfaces of many of the cells bear irregular, rounded, cytoplasmic projections. Most of the cells are of the mucous type, but interspersed among them are prominent eosinophilic cells. The epithelium rests upon a basement membrane which in turn is separated from the adjacent Leydig cells by a layer of collagenous fibers through which are interspersed a few longitudinally arranged smooth muscle cells. The ciliated epithelium forming the covering of the lower lip (fused internal palps) and the lining of the oral cavity is tall columnar with cilia decreasing in length from the entrance toward the oesophagus. Mucous cells are prominent, but are never distended to the extent of those of the upper lip. Dahmen (1923 (7)) found in *O. chilensis* that the mouth opening and oesophagus show a tall, cuticulated epithelium, upon which cilia could be demonstrated in only a few instances. Yonge (1926 (33)) observed that the epithelium of the mouth of *O. edulis* is continuous with

TABLE I

HISTOLOGICAL CHARACTERISTICS OF THE DIGESTIVE TRACT OF *Crassostrea virginica* (GMELIN)

(from specimens 8–10 cm. in length)

Division of tract	Epithelium						Extraepithelial tunics		
	Height of cell, μ	Length of cilia, μ	Cuticle	Mucous cells	Eosinophilic cells	Phagocytes	Basement membrane	Collag. conn. tissue	Smooth muscle
Palps									
Ridged surface	40–50	10–15	+	++	+	+	+	+	+
Smooth surface	20–30	5–7	±	+	+	+	+	+	+
Mouth									
Upper lip (fused external palps)	25–30	0–10	+	++	+	+	+	+	+
Lower lip (fused internal palps)	50–55	15–25	+	++	–	+	+	+	+
Roof	50–55	10–20	+	+	–	+	+	+	+
Floor	50–55	10–20	+	++	–	+	+	+	+
Oesophagus	75–105	10–15	+	++	+	+	+	+	+
Stomach									
Anterior chamber	55–180	15–20	+	+	–	+++	+	+	+
Caecum	20–155	7–15	+	+	–	+	+	+	+
Posterior chamber	45–180	15–25	+	+	–	+	+	+	+
Gastric shield region	50–230	–	+	–	–	++++	+	+	+
Style-sac, Mid-gut									
Style-sac	65–150	7–25	+	++	+	++	+	+	+
Mid-gut	55–160	5–20	+	+++	+	++++	+	+	+
Intestine									
Ascending limb	85–130	5–20	+	+	+++	++	+	+	+
Median limb	65–115	5–12	+	++	+	++	+	+	+
Descending limb	75–110	7–15	+	+++	+	++++	+	+	+
Rectum	50–125	15–25	+	+++++	+	+++	+	+	+
Anus	90	10	+	+++	+	++++	+	+	+
Digestive diverticula									
Ducts									
Large caecal and gastric									
Cell Type I	15–25	8–10	++	++	+	++	+	+	+
Cell Type II	25–40	4–8	+	++	+	++	+	+	+
Pretubular	10–25	4–6	+	+	+	+	+	+	+
Tubules									
Secretory cells	10–35	–	–	–	–	+	–	+	?
Generative cells	5–15	–	–	–	–	+	–	+	?

NOTE: + signs indicate presence and relative frequency.

that of the grooves between the palps and consists of long, ciliated cells with a few mucous glands, as did Leenhardt (1926 (17)) for *G. angulata.*

The epithelium of the oesophagus is tall, has a prominent cuticle, and bears heavy relatively short cilia, as in *G. angulata* (Leenhardt, 1926 (17)). Mucous cells are numerous and become more abundant toward the stomach, but eosinophilic cells are few. The basement membrane is heavy and lies on a distinct band of collagenous fibers containing a few strands of circularly and longitudinally arranged muscle cells. Phagocytes are scattered throughout the epithelium. Prominent folds may occur in the lateral margins of the oesophagus, which presumably would permit expansion during the passage of food to the stomach. Dahmen (1923 (7)) indicated a high, ciliated epithelium with some mucous cells for *O. chilensis*, as did Yonge (1926 (33)) for *O. edulis* where, however, phagocytes are numerous and "mucous" glands fewer.

Stomach (Figs. 21, 22, 32–34)

The internal surface of the stomach is thrown into irregular ridges separated by furrows. Epithelial cells covering the ridges are much taller than those lining the furrows, and such differences in height partially account for the irregular character of the lumen. With the exception of the gastric shield area of the posterior chamber, the entire epithelial lining is ciliated. Some mucous cells are present but few, if any, eosinophilic cells. In the caecum the epithelium progressively decreases in height from the point of exit from the anterior chamber to the blind terminations of the appendices, with the length of the cilia being correspondingly reduced. Phagocytes are present in large numbers below the prominent basement membrane, between the epithelial cells, and in the lumen. A few smooth muscle cells are arranged irregularly in both a longitudinal and circular direction to form, with collagenous fibers, an extraepithelial tunic. The gastric shield region appears in transverse section to be shaped like an arrowhead (Figs. 22, 23, 24). The epithelium resembles that of the other regions of the stomach except for the apparent lack of cilia. Fine cytoplasmic processes arising from the free borders of some of the cells unite with the overhanging gastric shield and are suggestive of degenerate or modified cilia (Fig. 35). The cells at the apex of the arrowhead (Fig. 36) are very tall, reaching a height of 230 μ, but taper laterally where they become continuous with the cellular lining of the adjacent posterior chamber. The nuclei are slender and situated somewhat nearer the basement membrane than in other regions of the stomach. Neither mucous nor eosinophilic cells appear to be present but numerous phagocytes occur between the epithelial cells. The histological structure of the stomach wall closely resembles that of other described species, *O. chilensis* (Dahmen, 1923 (7)), *G. angulata* (Leenhardt, 1926 (17)), and *O. edulis* (Yonge, 1926 (33)).

The gastric shield is composed of homogeneous layers of a chondroid-like substance (Nelson, 1918 (22)), which stains intensely with acid dyes. The laminated character is most readily exhibited in sections at the apex of the crest or ridge (Figs. 23, 24). The laminae gradually decrease in thickness from the apex to the margins of the shield. The hyaline gastric shields of

Mytilus (List, 1902 (18)) and of *Anodonta* (Gutheil, 1911 (15)) are formed by the fusion of droplets of secretion from the underlying epithelial cells. Although Yonge (1926 (33)) could find no evidence of such secretion in *O. edulis*, nevertheless the shield is joined to the epithelium by fine strands which pass through it and have the appearance of abortive fused cilia arising from basal granules. In *C. virginica*, as indicated above, there are evidences of cytoplasmic connections between the shield and the underlying epithelium and although, during the preparations of sections, such processes are usually severed near the apex, they can be demonstrated near the margins. Dahmen (1923 (7)) and Leenhardt (1926 (17)) consider the gastric shields of *O. chilensis* and *G. angulata* respectively to be products of cellular secretions which have filtered across the border of the epithelial cells to be incorporated with the cilia. The gastric shield could be analogous in certain respects to the peritrophic membrane of insects, which Wigglesworth (1950 (30)) has shown is in some instances composed of concentric lamellae secreted by the underlying epithelial cells of the mid-gut. It may be postulated, therefore, that if the gastric shield of the oyster is chiefly secreted by the epithelial cells beneath the apex, the chondrin would flow laterally, becoming attached to the cilia, which subsequently degenerate. Such a suggestion might account for the failure to observe any central attachment of the shield to the underlying epithelium, whereas the thinner margins invariably adhere by cytoplasmic processes.

Style-sac and Mid-gut (Figs. 25, 37, 38, 39)

The most striking feature of the epithelium lining the style-sac is the density and uniformity of the cilia in contrast to those found in any other region of the digestive tract. The cells are very regularly arranged with large ovoid nuclei. The internal fibrillar apparatus is more prominent than in any other part of the tract. The epithelium comprising the mid-gut wall varies considerably in height and the cilia lack the regularity of arrangement of those in the adjoining style-sac. Phagocytes and mucous cells are somewhat more numerous in the mid-gut wall than in that of the style-sac, (Table I) but eosinophilic cells are sparsely represented in both. In both style-sac and mid-gut the basement membrane is surrounded by a layer of collagenous fibers with sparsely interspersed circularly arranged smooth muscle fibers. Dahmen (1923 (7)), Yonge (1926 (33)), and Leenhardt (1926 (17)) describe no muscle cells around the epithelium in *O. chilensis*, *O. edulis*, and *G. angulata*, but otherwise this division of the tract appears to be essentially the same in these species.

The typhlosoles consist of a core of Leydig cells covered by tall, columnar epithelial cells which grade on one side into those of the style-sac, and on the other into those of the mid-gut. However, the transition from the epithelium of the style-sac to that of the mid-gut is much more abrupt for the smaller or right typhlosole than for the larger or left one and is usually marked by a cleft. The cilia of the smaller typhlosole approximate those of the style-sac in length measuring up to 20 μ while those of the larger typhlosole

range from 10 to 15 μ. Mucous cells are usually more abundant on the larger typhlosole.

The lumen of the style-sac is occupied by a gelatinous white to yellowish-brown rod, the crystalline style, whose anterior end extends into the posterior chamber of the stomach to rest against the gastric shield. The central core is fluid while the periphery is firm and consists of several gelatinous strata. In fixed specimens the style usually disintegrates, but occasionally its outer layers may be identified in sections (Figs. 22, 34). There are diverse opinions regarding the origin of the acellular crystalline style in lamellibranchs. Its formation has been attributed to the secretory activity of the walls of the "caecum" (Barrois, 1889 (2)) and the "liver" (Mitra, 1901 (21)). Although many investigators (List, 1902 (18); Nelson, 1918 (22); Edmondson, 1920 (8); Mackintosh, 1925 (20); Graham, 1930 (13)) have suggested that it is secreted by the narrow cells of the minor or right typhlosole, none have been able to offer authentic evidence. Gutheil (1912 (16)) has described clear secretory granules above the nuclei in the ciliated cells of the style-sac of *Anodonta cellensis*. Yonge (1926 (32)) demonstrated the presence of droplets of secretion in the style-sac epithelium of part of the minor typhlosole of both *Mytilus edulis* and of *O. edulis* after the injection of iron saccharate, and concluded that these cells of the groove of the style-sac are instrumental in the formation of the style. Leenhardt (1926 (17)) observed certain haematoxylin-staining granules in the style-sac ("caecum") of *G. angulata* to which he attributed the secretion of the crystalline style. There has been no evidence of such specifically staining granules in the epithelium of the style-sac of *C. virginica*.

Intestine—Ascending, Median, and Descending Limbs (Figs. 25, 26, 41, 42)

The intestine is characterized by a prominent typhlosole with a median groove. The narrow lumen has the form of a double crescent when observed in transverse section. The epithelial lining exhibits great uniformity throughout all three limbs, the ciliated cells resembling those of the mid-gut adjoining the style-sac. Mucous cells and eosinophilic cells are both present, with the former increasing in abundance along the course of the tract (Table 1). Phagocytes are numerous among the epithelial cells, below the basement membrane, as well as in the lumen of the tract. The basement membrane of the ascending limb is very broad, but becomes progressively less pronounced as the intestine grades into the rectum. It rests upon a thin layer of collagenous fibres among which are occasionally interspersed a few smooth muscle fibers, most of which are circularly arranged.

The typhlosole consists of a more or less V-shaped mass of Leydig cells surmounted by epithelial cells which are tallest on either side of the median groove, and becomes lowest at the base of the groove. On the opposite wall of the gut, the epithelium in the mid-line may project into the median groove of the typhlosole.

Morphologically and histologically, the intestine resembles that described by Dahmen (1923 (7)) for *O. chilensis* and by Yonge (1926 (33)) for *O. edulis* except that in these species muscular tissue has not been demonstrated. Field (1922 (11)) describes circularly arranged muscle fibers in the intestine of *Mytilus edulis* while Leenhardt (1926 (17)) illustrates for *G. angulata* an epithelium comparable to that of *C. virginica*, but does not refer to any muscular cells although the connective tissue layer is prominent. He is in agreement with Sabatier (1887 (27)), who suggested that the connective tissue layer of the intestine of *Mytilus edulis* could be considered as a lymphoid organ because of the large number of amoebocytes present. Such a condition may also hold true for *C. virginica* since these cells are abundant in this region of the tract.

Rectum (Figs. 27, 28, 43, 44)

The rectum resembles the preceding limbs of the intestine with the exception that the typhlosole is more pronounced and the median groove correspondingly deeper. Numerous epithelial folds occur in the lateral walls of the rectum while coarser folds appear in the adjacent typhlosole. The typhlosole gradually disappears near the anal region when the tract assumes a more or less circular outline. Its internal surface is thrown into small folds.

There is a marked increase in the numbers of mucous cells progressively from the median limb of the intestine toward the rectum and anus. Sometimes they may even predominate in numbers over the ciliated cells. Relatively few eosinophilic cells occur in either the rectum or the anal region. Phagocytic cells are numerous throughout the epithelium and beneath the thin basement membrane. There is a definite layer of smooth muscle fibers, circularly arranged, in the region of the anus. Leenhardt (1926 (17)) noted smooth muscle fibers in *G. angulata* which became sufficiently abundant in the anal region as to form a sphincter, but neither Dahmen (1923 (7)) nor Yonge (1926 (33)) refer to them in *O. chilensis* and *O. edulis*.

Digestive Diverticula (Figs. 15, 16, 29, 45, 46, 47)

The digestive diverticula in the oyster are organs of absorption and of intracellular digestion (Yonge, 1926 (32)). The tubules and the ducts of these diverticula are grouped in the form of small lobules indistinctly separated and bound together by interlobular connective tissue composed of Leydig cells with some collagenous fibers. The histological structure of the larger ducts which communicate directly with the caecum and the posterior stomach generally resembles the adjacent region of the stomach. The lumina sometimes appear circular in outline, at other times crescentic or often multiangular. As the primary ducts branch leading toward the secretory tubules, a difference in the height and character of the ciliated epithelium occurs on opposite sides of the wall. On one side the cells are somewhat short and broad with lightly staining nuclei (Cell Type I, Table I). There is a heavy cuticular border and the cilia are relatively long and dense. Cells on the opposite side may be almost double in height, the cuticle is thin, and the cilia

somewhat shorter (Cell Type II, Table I.). Mucous cells are much more profuse among the elongated ciliated cells, and eosinophilic cells occasionally occur. The lumina of the immediate pretubular ducts are generally circular in outline and the epithelial cells become progressively uniform in height. Phagocytes are present in the epithelium, in the lumina, and in the surrounding connective tissue. Circularly arranged smooth muscle fibres imbedded in collagenous connective tissue surround the basement membrane. The structure of the duct resembles that described by List (1902 (18)) and Field (1922 (11)) for the "liver canals" of the digestive diverticula of *Mytilus edulis*. Dahmen (1923 (7)) and Yonge (1926 (33)) observed no special variation in the epithelium of the ducts from that of the stomach for *O. chilensis* and *O. edulis* respectively, but Leenhardt (1926 (17)) recorded an arrangement for *G. angulata* which is similar to that observed here in C. *virginica*.

The histological structure of the secretory units of the digestive diverticula is quite different from that of the ducts and a seasonal variation is exhibited. Yonge (1926 (32)) has described the units as tubules for a large number of lamellibranchs including *O. edulis*, as did Dahmen (1923 (7)) for *O. chilensis*, while Leenhardt (1926 (17)) has considered them to be in the form of acini in *G. angulata*. Graphic reconstruction of the units from serial sections indicates their tubular rather than their acinar character in *C. virginica*. During the spring and summer months when the lining epithelium is tall, the lumina of the tubules appear roughly in the form of a cross or H and less frequently tripartite. The non-ciliated epithelium comprising the wall is of two types. Generative cells located in the crypts formed by the extremities of the cross are small with large nuclei, each of which possesses a nucleolus. The cytoplasm is somewhat granular and darkly-staining and a basement membrane is not evident. Both Yonge (1926 (32)) and Leenhardt (1926 (17)) consider that these cells are capable of replenishing the absorptive and secretory cells of the remainder of the tubule. Cells of the latter type progressively increase in height from the crypt toward the central lumen and rest on a basement membrane. The cytoplasm is coarsely vacuolated and stains very lightly in comparison with that of the generative cells. Sometimes engulfed food particles are evident. Phagocytes may be present among the epithelial cells. A thin layer of collagenous connective tissue surrounds each tubule. During the winter season (Fig. 47) the lumina of the majority of the tubules become spherical or somewhat ovoid in outline. The peripheral borders of the large coarsely vacuolated cells are quite irregular and some exhibit extensive degeneration. There appears to be no change in the generative cells which occur at the angles of the crypts.

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FIGS. 17–29. Photomicrographs of histological sections of the digestive tract of *Crassostrea virginica*.

FIG. 17. Longitudinal section of the adjacent ridged medial surface of an outer labial palp, and the lateral surface of an inner labial palp. Ehrlich's haematoxylin and Triosin, 10 μ. ×50.

FIG. 18. Longitudinal section of a ridge and adjacent furrows of the lateral surface of an inner labial palp showing the almost straight proximal wall and the grooved distal wall. Ehrlich's haematoxylin and Triosin, 10 μ. ×300.

FIG. 19. Sagittal section showing the relationships of the labial palps, mouth cavity, and oesophagus. Mallory's triple stain, 10 μ. ×12.

D.D., digestive diverticula; I.P., inner palp; M., mouth cavity; O., oesophagus; O.P., outer palps.

FIG. 20. Sagittal section of oral cavity showing low non-ciliated and sparsely ciliated epithelium of upper lip (fused external palps) merging into the typical ciliated columnar epithelium on the roof and floor (right). Mallory's triple stain, 10 μ. ×85.

FIG. 21. Slightly oblique transverse section of stomach region of 5 mm. oyster spat. A.S., anterior chamber of stomach; C., caecum; D.D., digestive diverticula; P.S., posterior stomach. Alum cochineal and light green. ×100.

FIG. 22. Coronal section through the stomach. ×12.

A.S., anterior chamber of stomach; C., caecum and (anterior appendix); C.S., crystalline style; D.D., digestive diverticula; G.S., gastric shield; P.S., posterior stomach. Ehrlich's haematoxylin and Triosin.

FIG. 23. Posterior chamber of stomach showing cliplike attachment of gastric shield to underlying epithelium. Ehrlich's haematoxylin and Triosin, 10 μ. ×33.

FIG. 24. Enlarged view of Fig. 23 showing the laminated character of the gastric shield. ×150.

FIG. 25. Cross section of the style-sac and mid-gut, and ascending limb of intestine. Ehrlich's haematoxylin and Triosin, 10 μ. ×20.

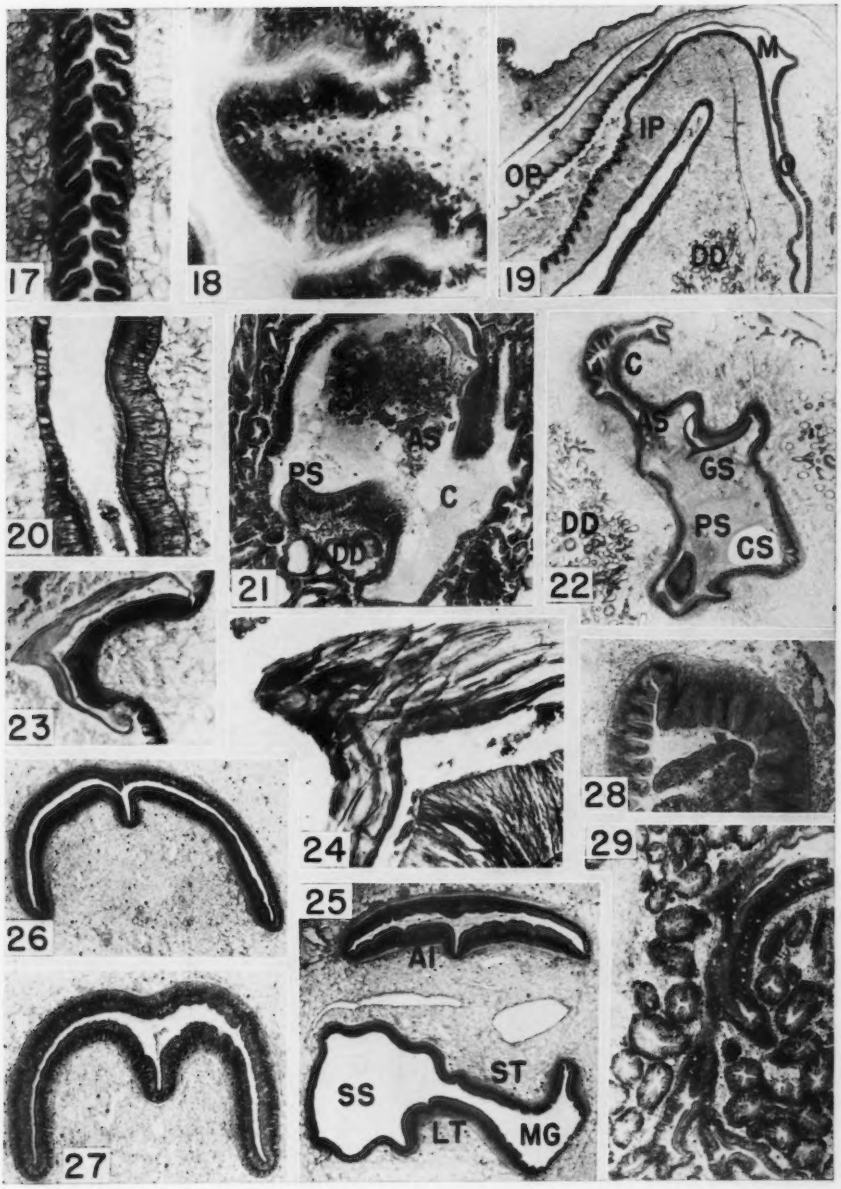
A.I., ascending limb of intestine; M.G., mid-gut; S.S., style-sac; L.T., larger or left typhlosole; S.T., smaller or right typhlosole.

FIG. 26. Transverse section of the descending limb of the intestine showing the prominent typhlosole with the median groove. Van Gieson's and light green, 10 μ. ×20.

FIG. 27. Transverse section of the rectum showing pronounced grooved typhlosole. Ehrlich's haematoxylin and Triosin, 10 μ. ×25.

FIG. 28. Transverse section of preanal rectum; the typhlosole is disappearing, epithelial folds are present. Ehrlich's haematoxylin and Triosin. ×35.

FIG. 29. Portion of an extensively branched caecal duct of the digestive diverticula with sections of adjacent tubules. Ehrlich's haematoxylin and Triosin, 10 μ. ×70.



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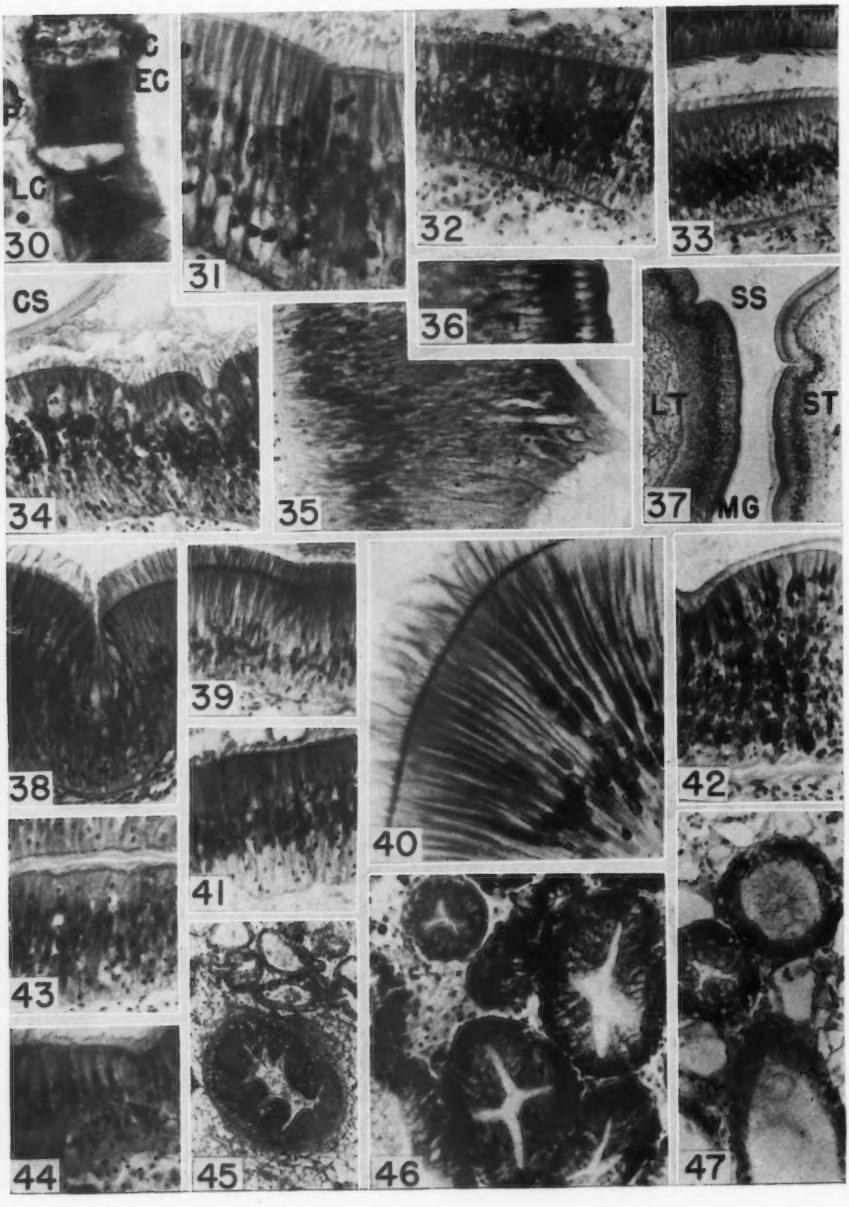

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FIGS. 30–47. Photomicrographs of histological sections of the digestive tract of *Crassostrea virginica*.

FIG. 30. Non-ciliated and sparsely ciliated epithelium of the upper lip (fused external palps). Mallory's triple stain, 10 μ. ×600.

E.C., eosinophilic cell; L.C., Leydig cell; M.C., mucous cell; P., phagocyte or leucocyte.

FIG. 31. Ciliated epithelial lining of oesophagus. Ehrlich's haematoxylin and Triosin, 7 μ. ×450.

One prominent eosinophilic cell is evident, also numerous mucous cells and phagocytes.

FIG. 32. Wall of the anterior chamber of stomach. Ehrlich's haematoxylin and Triosin, 10 μ. ×250.

The ciliated cells have a prominent cuticular border. Mucous cells are abundant and phagocytes occur in the lumen and throughout the epithelium and underlying layers.

FIG. 33. Mid-region of the anterior appendix of the caecum. Ehrlich's haematoxylin and Triosin, 10 μ. ×250.

FIG. 34. Wall of the posterior chamber of stomach. Ehrlich's haematoxylin and Triosin, 10 μ. ×225.

Phagocytes are numerous throughout the epithelium. The cilia are long and the cuticular border is prominent. Mucous cells are abundant. C.S., crystalline style.

FIG. 35. Wall of the posterior chamber of stomach immediately under the apex (neck) of the gastric shield (G.S.). Ehrlich's haematoxylin and Triosin, 10 μ. ×225.

The gastric shield has separated from the surface of the epithelial cells during sectioning. The non-ciliated epithelial cells are the maximum height for the digestive tract.

FIG. 36. Marginal area of the gastric shield showing the cytoplasmic processes of attachment to the peripheral borders of the cells. Iron haematoxylin and light green, 7 μ. ×500.

FIG. 37. Junction of style-sac and mid-gut. Ehrlich's haematoxyli nand Triosin, 10 μ. ×75.

L.T., larger or left typhlosole; M.G., mid-gut; S.S., style-sac; S.T., smaller or right typhlosole.

FIG. 38. Enlarged view of style-sac on right and adjoining smaller typhlosole of mid-gut on left. ×225.

FIG. 39. Longitudinal section through the style-sac showing uniformity of heavily ciliated surface. Few phagocytes are present. Ehrlich's haematoxylin and Triosin, 10 μ. ×200.

FIG. 40. Typical ciliated epithelium of the style-sac showing internal fibrillar apparatus. Cresyl echt violet, 7 μ. ×500.

FIG. 41. Longitudinal section through the mid-gut showing ciliated epithelial cells and abundant phagocytes. Ehrlich's haematoxylin and Triosin, 10 μ. ×200.

FIG. 42. Transverse section of the ciliated epithelial lining of the ascending limb of intestine. Ehrlich's haematoxylin and Triosin, 7 μ. ×225.

Eosinophilic cells are darkly stained. Mucous cells and phagocytes are numerous.

FIG. 43. Cross section through the ciliated epithelial lining of the lateral wall and adjacent typhlosole of the rectum. Phagocytes are present in large numbers. Ehrlich's haematoxylin and Triosin, 7 μ. ×225.

FIG. 44. Coronal section of ciliated epithelial surface of the anal region, showing prominent mucous cells and infiltration of phagocytes. Ehrlich's haematoxylin and Triosin, 10 μ. ×225.

FIG. 45. Transverse section of a large duct of the digestive diverticula with some degenerating tubules from a specimen fixed during the early winter (late November). Ehrlich's haematoxylin and Triosin, 10 μ. ×70.

Mucous cells interspersed among the ciliated epithelial cells are abundant in the upper two-thirds of the duct but are rare in the lower third where the cuticle is heavy.

FIG. 46. Typical tubules of the digestive diverticula with large lightly-staining vacuolated secretory and absorptive cells, and small darkly-staining generative cells. A section of a small duct to the upper left. Ehrlich's haematoxylin and Triosin, 10 μ. ×275.

FIG. 47. Typical tubules of the digestive diverticula during the winter season, showing the degeneration of the free border of the vacuolated secretory cells. Ehrlich's haematoxylin and Triosin, 10 μ. ×275.

LIFE CYCLE AND MORPHOLOGY OF PARUTERINA RAUSCHI N.SP. and P. CANDELABRARIA (GOEZE, 1782) (CESTODA) FROM OWLS, AND SIGNIFICANCE OF PLEROCERCOIDS IN THE ORDER CYCLOPHYLLIDEA[1]

REINO S. FREEMAN

Abstract

Paruterina rauschi n.sp. is described from the barred owl, and *P. candelabraria* (Goeze, 1782) is redescribed from the snowy owl; both species grow in the great horned owl. The life history and development of the plerocercoid of both species of worms in various rodents is described. Natural infections with the plerocercoid of *P. rauschi* n.sp. are reported from *Tamias striatus* and *Peromyscus leucopus*. Plerocercoids are not uncommon in cyclophyllidean life cycles, and their significance in the taxonomy of the order Cyclophyllidea is discussed. It is concluded that any future taxonomic revision of this order must consider the morphology of the immature stages if such a revision is intended to clarify natural relationships.

Larval cestodes, or metacestodes (23), which occur in the viscera of a variety of small mammals, frequently are difficult to identify. A few are described from known material obtained from life history studies, and some, because of distinctive scoleces, can be associated with known adults. Some are not yet described, and descriptions of others are inadequate. Further confusion results from the use of the terms cysticercus and cysticercoid for larvae resembling plerocercoids. Larvae of the plerocercoid type, which could not be associated with any described adults or larvae, have been encountered in Minnesota and Ontario in the past several years. A suggestion from Dr. Robert Rausch, Arctic Health Research Center, Anchorage, Alaska, led me to suspect that some of these plerocercoids might be those of *Paruterina* sp. from owls. Study of adult worms from several geographical areas, and several species of owls, as well as plerocercoids from life history studies, reveals that two species of *Paruterina* occur in North American owls. The adult and plerocercoid, as well as observations on the life history, of *Paruterina rauschi* n.sp. and *P. candelabraria* (Goeze, 1782) are described herein.

Materials and Methods

Rodents and owls were examined as described previously (5). Most animals were examined soon after death, although a few owls had been frozen prior to the examination. Living plerocercoids showed most detail. Worms were fixed in cold or hot 10% formalin or F.A.A. solution, and stained with Mayer's hemalum or carmalum. Permanent mounts of rostellar hooks from adults and plerocercoids were made by removing the rostellum with its hooks, flattening it under cover glass, and clearing and dehydrating it in beechwood

[1]Manuscript received January 17, 1957.
Contribution from Department of Parasitology, Ontario Research Foundation, Toronto, Ontario, Canada.

creosote. Plerocercoids were sectioned and stained with haematoxylin and eosin. Adults and plerocercoids from several parts of the United States were examined through the kindness of several workers. Some hosts for experimental studies were reared in captivity, while others were trapped alive in the field. Cestode eggs were fed to rodents by mixing the contents of one or more proglottides with the food, or by directly inserting proglottides into the stomach with a fine polyethylene tube mounted on a 5 cc. syringe. Owls were fed cestode larvae either *in situ* in the host, or removed from the host cyst and placed among the viscera of a rodent. Owl feces were concentrated by the usual screening and sedimenting technique, but apparently eggs of *Paruterina* are not released into the feces by the paruterine organ, and no eggs or proglottides were found.

Description of Adults

Paruterina ***rauschi*** n.sp.

Synonym: *Paruterina candelabraria* (Goeze, 1782), in Rausch (16), *partim*

Hosts: *Strix varia* (barred owl)
Bubo virginianus (great horned owl)
Aegolius acadica (?) (saw-whet owl)

Distribution: Southern Canada and United States

Intermediate hosts: Various rodents

Diagnosis

Paruterinidae Skrjabin, 1940; Paruterininae Fuhrmann, 1907; *Paruterina* Fuhrmann, 1906. Strobila, 238 mm. in length by 1.06 mm. in width (195 by 1.14 mm. to 238 by 1.06 mm.),* with 583 (507 to 634) proglottides. Scolex 150 μ in length by 225 μ in width (150 by 225 μ to 153 by 201 μ); suckers 76 by 81 μ to 89 by 94 μ (68 by 76 μ to 89 by 94 μ); rostellar pad difficult to measure on holotype, about 62 μ in length by 111 μ in width (60 by 81 μ to 69 by 87 μ). About 50 rostellar hooks on holotype (41 to 50), large hooks 42 μ in length (39 to 42 μ), small hooks 32 μ in length (30 to 33 μ) (Fig. 1). Region of sexual maturity† about proglottis No. 365 (365 to 385). Excretory ducts in holotype as in Fig. 3, but dorsal duct may be more lateral in position in some specimens. Genital pores, irregularly alternate and anterior to middle on lateral margin of proglottis, open into shallow genital atrium. Testes, numbering from 27 to 32 (26 to 32), ovoid to irregular in shape, measure from 35 by 54 μ to 44 by 69 μ (35 by 54 μ to 42 by 72 μ), and occur up to three testes deep behind and along each side of female genital complex; in some cases testes may overlap excretory vessels, but not beyond the lateral margin of ventral excretory duct (Fig. 3). Cirrus pouch varies from 174 μ

*Measurement from holotype followed in parentheses by minimum and maximum measurements from three paratypes; wherever possible five measurements were taken, but 20 or more are included in most cases. Additional observations were made on strobilar fragments.

†The region of sexual maturity is considered to begin at that point in the strobila where a cavity appears in the uterine primordium. In well-fixed material the nuclei in the primordium appear as a ring of dots dorsal and usually slightly anterior to the ovarian isthmus (Figs. 3 and 4).

in length by 56 μ in width to 200 μ by 50 μ (139 μ by 71 μ to 200 μ by 50 μ) when cirrus not everted, with proximal end extending well beyond inner margin of poral ventral excretory duct. One everted cirrus, unarmed, measured 180 by 28 μ. In some specimens the cirrus of postmature proglottides inserted into vagina of same proglottis. Ejaculatory duct convoluted within cirrus pouch and continuous with highly convoluted vas deferens (Fig. 3). Vagina posterior and slightly ventral to cirrus pouch, about 390 μ in length and 28 μ in maximum width, terminates proximally in a relatively small, elongate seminal receptacle near the ovarian isthmus. Vagina with glandular cells along most of its length and with a sphincter just before opening into genital atrium. Ovary bilobate up to 300 μ in transverse dimension with each lobe from 42 by 118 μ to 69 by 139 μ (65 by 97 μ to 37 by 164 μ). Vitelline gland transversely elongate but irregular in shape, from 56 by 132 μ to 50 by 168 μ (42 by 99 μ to 37 by 164 μ), with the Mehlis gland, usually poorly stained and difficult to differentiate, in its anterior face. Uterus begins as a slightly more densely staining area dorsal to ovarian isthmus and becomes hollow in mature proglottides. In semiripe proglottides, uterus a transverse sac more posteriorly located in proglottis with developing paruterine organ anterior to it. Fully developed paruterine organ not found in proglottides attached to complete strobila. Embryonated eggs 25 by 19 μ to 27 by 20 μ when fixed.

Holotype from great horned owl, and paratypes from barred owl and great horned owl, along with plerocercoids from house mice, deposited in the U.S. National Museum.

Paruterina candelabraria (Goeze, 1782) Fuhrmann, 1906

Synonyms: *Taenia candelabraria* Goeze, 1782
Taenia candelabraria of Krabbe (12) and Wolffhügel (24)

Hosts: In Europe—various species of owls (24)
In North America—*Nyctea scandiaca* (snowy owl)
Bubo virginianus (great horned owl)
Asio otus wilsonianus (long-eared owl)
Aegolius acadica (saw-whet owl)

Distribution: In northern hemisphere, northern United States, Canada, Europe, and Asia (?)

Intermediate hosts: Various rodents

Diagnosis

Paruterinidae Skrjabin, 1940; Paruterininae Fuhrmann, 1907; *Paruterina* Fuhrmann, 1906. Strobila from 112 mm. in length by 1.08 mm. in width to 203 by 1.4 mm., with 579 to 740 proglottides.* Scolex from 231 μ in length by 241 μ in width to 254 by 300 μ; suckers from 92 by 72 μ to 132 by 125 μ;

*Six complete worms and 10 isolated scoleces measured; additional observations on isolated strobilar fragments.

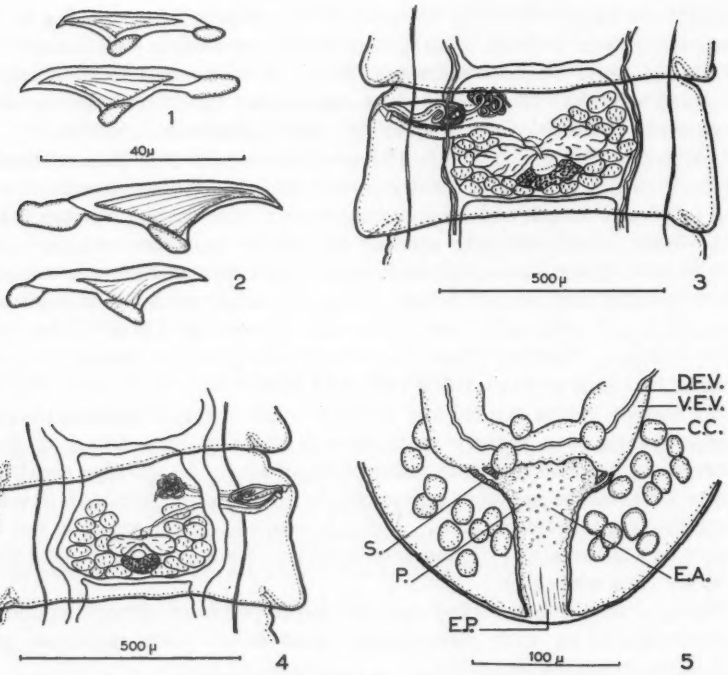


FIG. 1. Large and small rostellar hooks of *Paruterina rauschi* n.sp.
FIG. 2. Large and small rostellar hooks of *P. candelabraria*.
FIG. 3. Mature proglottis of *P. rauschi* n.sp.
FIG. 4. Mature proglottis of *P. candelabraria*.
FIG. 5. Posterior tip of plerocercoid of *P. rauschi* n.sp. (same plerocercoid as in FIG. 9). C.C., calcareous corpuscle; D.E.V., dorsal excretory vessel; E.A., excretory atrium; E.P., excretory pore; P., papilla; S., sphincter; V.E.V., ventral excretory vessel.
FIGS. 1 and 2 drawn with the aid of a *camera lucida*, FIGS. 3 and 4 with the aid of a microprojector, and FIG. 5 drawn from a photomicrograph.

rostellar pad from 72 by 108 μ to 42 by 143 μ. From 39 to 46 rostellar hooks, large hooks from 49 to 56 μ in length, small hooks from 36 to 42 μ in length (Fig. 2). Region of sexual maturity from proglottis No. 450 to No. 490, general distribution of organs as in Fig. 4. Excretory ducts and genital pores are as described for *P. rauschi*. Testes, numbering from 21 to 39, mostly ovoid, measuring from 35 by 44 μ to 56 by 83 μ, two to three testes deep behind and along both sides of the female genital complex, rarely some testes may just overlap medial margin of ventral excretory duct. Cirrus pouch varies from 90 by 49 μ to 125 by 49 μ in maximum dimensions with proximal end only rarely beyond the lateral margin of poral ventral excretory duct. Cirrus difficult to measure. Ejaculatory duct convoluted within cirrus pouch and continuous with convoluted vas deferens. Vagina like that in *P. rauschi*, varies from 300 by 20 μ to 400 by 35 μ in dimensions. Ovary bilobate from 150 μ up to 210 μ in maximum transverse dimension with each lobe from 25 by 58 μ to 87 by 110 μ. Vitelline gland transversely elongate from 29 by

85 μ to 62 by 132 μ with Mehlis gland in its anterior face. Uterus begins and develops as described for *P. rauschi*. Fully developed paruterine organ not found in proglottides attached to complete strobila. Embryonated eggs when fixed 33 by 21 μ to 35 by 24 μ.

Slides of worms from a snowy owl and a great horned owl, as well as plerocercoids from house mice, deposited in U.S. National Museum.

P. rauschi n.sp. differs from *P. otidis* Baczynska, 1914 and *P. angustata* Fuhrmann, 1906, two other species reported from owls, in that *P. angustata* has unilateral genital pores, has 20 testes, is only about 50 mm. in length, and has hooks of different shape, whereas *P. otidis* is even smaller (20 mm.), has fewer testes (15), and has 92 hooks. The rostellar hooks of the latter two are larger, being about the size of those on *P. candelabraria*. *P. rauschi* n.sp. differs from *P. candelabraria* in the following respects. The large rostellar hooks of *P. rauschi* are only about the size of the small rostellar hooks of *P. candelabraria*. The hooks of the two species are similar in fundamental morphology, but the guard of the large hooks of *P. candelabraria* is heavier and the tip rounded compared with the angular appearance in *P. rauschi* (Figs. 1 and 2). The region of sexual maturity occurs more anteriad (proglottis No. 310 to 385) in *P. rauschi* than in the other (No. 400+ to 565). The cirrus pouch is smaller in *P. candelabraria* and does not cross the inner margin of the poral ventral excretory vessel, whereas the proximal end of the larger cirrus pouch of *P. rauschi* extends beyond this margin. The ovary and vitelline gland are larger in *P. rauschi*, although specific measurements of one may overlap those of the other species. Fixed eggs of *P. rauschi* are somewhat smaller than those of *P. candelabraria*.

The earliest basis for recognizing *P. candelabraria* (Goeze, 1782) was its external appearance and its occurrence in owls (6, 19, 20). The presence of hooks on this species was first established by Mehlis (14). It is the hooks as figured by Krabbe (12), however, which Wolffhügel (24) and later workers accept as most characteristic of the species. Up to the year 1900 all cestodes from owls in Europe were assigned to *Taenia candelabraria*. *Taenia strigis acadicae* Leidy, 1855, from a North American owl was inadequately described, and is probably a *nomen dubium*. Wolffhügel (24) gave the first detailed description of the internal anatomy of a worm, which he identified as *T. candelabraria*, from the owl *Asio otus* (L.) (= *Otus vulgaris* Flemm.). Unfortunately, there was no scolex on the specimen that was described and conceivably it was not *P. candelabraria*, although the internal morphology of *P. candelabraria*, as understood today, is based on this description. Verification of this description requires re-examination of hook-bearing specimens from European owls, since three other species of *Paruterina* from owls are known to exist. Wolffhügel's (24) description of *P. candelabraria* differs from that presented here in one significant point, namely, the cirrus pouch he describes is larger than on my specimens and in the schematic drawing of the mature proglottis Wolffhügel shows the proximal end extending appreciably beyond the inner margin of the poral ventral excretory vessel.

Since the specimens described in the current study as *P. candelabraria* have hooks which are identical in morphology and similar in number with those described by Krabbe (12), it is assumed that the North American form is correctly identified.

Rausch (16) recovered a series of cestodes from several species of owls from North America; some of them lacked hooks. He compared his material with specimens of *P. candelabraria* identified by Fuhrmann from European owls, and claimed that they agreed with each other in most respects, as well as he could tell from Fuhrmann's old specimens. Rausch found the European material had large hooks, averaging 56 μ, whereas his own specimens averaged 43 μ, but he felt this difference in size was not exceptional, and he says: ". . . there is considerable variation in hook size according to Wolffhügel (1900)". Wolffhügel (24), however, had no scoleces for study and only recorded Krabbe's (12) observations that *P. candelabraria* has 40 hooks, the larger 54 μ in length and the smaller 35 to 37 μ in length. Later workers, e.g. Joyeux and Baer (10), repeat those exact measurements leaving the impression that variation in hook size is limited rather than considerable. The hooks described by Rausch agree completely with those of *P. rauschi* as described here, suggesting the possibility that his description is that of *P. rauschi*. Unfortunately, some of the other measurements which he included in his description overlap both *P. candelabraria* and *P. rauschi* described above. Further, his specimen No. 46327, deposited in the U.S. National Museum, from a great horned owl collected from the north central United States is clearly *P. candelabraria* (Table I) as he said. Rausch presumably had both species before him, but apparently his specimens of *P. candelabraria* lacked hooks.

Recently Mahon (13) reported *Paruterina candelabraria* from the saw-whet owl and barred owl from Canada. She assumed the worms from the two owls are the same species, and gave a few measurements. The hooks she described agree in size and morphology with *P. candelabraria* from Europe, and as described here. Since there was a difference in the shape of the guard on hooks on her specimens and those drawn by Rausch, she postulated that perhaps he had drawn damaged hooks. Mahon accepted Rausch's statement, however, that the hooks of this species show considerable variation in size and considered her material cospecific with *P. candelabraria* from Europe and the specimens as redescribed by Rausch. I re-examined the specimens from the barred owl studied by Mahon, but could not identify the species since no scoleces and only postmature strobilar fragments were present. The specimens from the saw-whet owl, however, were very perplexing. Two isolated preparations of scoleces had hooks, which are identical with those of *P. candelabraria* (Table I). Two other slides contained more or less complete strobilae, but the scoleces lacked hooks. Measurements from the longest and best specimen are included in Table I. The specimen differed from any encountered in this study as the region of sexual maturity occurred in about proglottis No. 200; in fact the terminal proglottis, No. 272, had an extensive uterus filled with eggs. The worm was only 31 mm. in length, and, except for the scolex, was

TABLE I

MEASUREMENTS OF *Paruterina* SPP. FROM OWLS FROM VARIOUS LOCALITIES

	U.S.N.M. No. 46327 North Central States	U.S.N.M. No. 30242 Montana	U.S.N.M. No. 30243 Montana	Hoffman North Dakota	Mahon Slide No. 1 Quebec	Mahon Slide No. 5 Quebec	Hoffman Iowa	Schiller Maryland
Host	Great horned owl	Great horned owl	Long-eared owl	Snowy owl	Saw-whet owl	Saw-whet owl	Barred owl	Barred owl
No. of worms examined	1	1	1	2	1	1	1	2
Fix	?	?	?	Bouin's	?	?	Bouin's	?
Length-width of scolex, μ	219 × 162	266 × 277	318 × 347	118 × 132		285 × 214	196 × 185	202 × 196
Size of suckers, μ	101 × 61	103 × 93	*Ca.* 140	49 × 76 to 58 × 50	90 × 97 to 104	118 × 78 to 132 × 90	74 × 78 to 83 × 62	97 × 83 to 103 × 76
Rostellar pad, μ	86 × 100	42 × 140	104 × 140	81 × 76	*Ca.* 60 × 143	75 × 118	69 × 64	76 × 97
Length of large hooks, μ	Missing	51+	50 to 56	55 to 56	56	Missing	Missing	Missing
Length of small hooks, μ	Missing	37+	43 to 45	42 to 44	44 to 46	Missing	Missing	Missing
Length of "neck", mm.	2.6	0.4	1.9	1.6 to 1.7	*Ca.* 1.1	0.26	2.6	2.3
Region of sexual maturity	565	445	*Ca.* 440	*Ca.* 400+		*Ca.* 200	Not mature at 240	310 to 370
Length–width of cirrus pouch, μ	139 × 56	132 × 49	?	111 × 36 to 111 × 44		146 × 62 to 140 × 78	139 × 76	150 × 65 to 153 × 76
Position of ental end of cirrus pouch	Does not reach inner margin of v.e.v.	May reach lateral margin of v.e.v.	Can't tell	Maximum of half way between margins of v.e.v.		Can't tell for sure, probably overlaps	Extends far beyond inner margin of v.e.v.	At least reaches inner margin of v.e.v. or extends beyond
No. of testes	?	28	Can't tell	25 to 28		27 to 31	28	?
Diameter of testes, μ	39 × 42 to 50 × 39	44 × 50 to 53 × 56	Can't tell	39 × 42 to 51 × 46		21 × 49 to 28 × 56	35 × 32 to 46 × 56	35 × 56 to 50 × 50
Size of one lobe of ovary, μ	56 × 76 to 62 × 97	60 × 72 to 54 × 86	Can't tell	69 × 74 to 90 × 53		*Ca.* 28 × 170	93 × 87	70 × 140 to 76 × 181
Size of vitelline gland, μ	35 × 111	46 × 92	Can't tell	42 × 79 to 56 × 95		28 × 145	56 × 128	56 × 163 to 65 × 167
Identification	*P. candelabraria*	*P. candelabraria*	*P. candelabraria*	*P. candelabraria*	*P. candelabraria*	*P. rauschi*	*P. rauschi*	*P. rauschi*

much smaller than any other worm in this study. The small size may be attributed, in part, to the method of fixation (unknown). The only other specimens, however, which remotely resembled Mahon's specimens in size were those Hoffman fixed in Bouin's fixative (Table I). In other respects, however, Hoffman's material was readily identified to species. Mahon's specimens most nearly fit the description of *P. rauschi*, and that is the tentative identification made here. Therefore, the saw-whet harbored both species of *Paruterina*, which differs from the condition found in other owls.

Development of the Paruterine Organ

Fully ripe proglottides of *P. candelabraria* were not seen during the present study, although proglottides with eggs were present. Free proglottides of *P. rauschi* including fully ripe ones were examined, however, so the development of the uterus and paruterine organ in *P. rauschi*, as observed in whole mounts, is described.

The uterus becomes visible first as a darker staining area dorsal and occasionally slightly anterior to the ovarian isthmus. At the time when all other sexual organs are clearly delineated, a hollow, the beginning of the uterine cavity, becomes visible in the uterine primordium. Shortly after this the primordium of the paruterine organ appears as a darker staining area reaching from the uterus toward the anterior end of the proglottis. In successive proglottides the uterine cavity enlarges, primarily along a transverse axis dorsal to the ovary. Ultimately, the uterus becomes as wide as the ovary, by which time the ovary is difficult to differentiate. Eggs are now apparent in the uterus. Meanwhile the primordium of the paruterine organ has become more pronounced and reaches from the uterus to the anterior end of the proglottis as a broad band of more intensely staining substance. Further development for an extended length of the strobila consists of growth of the uterus, which frequently becomes bilobate with a longitudinal cleft extending anteriorly from the posterior face; more eggs appear in the uterus. Before the uterus is fully developed the primordium of the paruterine organ becomes more compact, and then in successive proglottides it is first sinuous and finally develops a series of bends; more bends appear to develop in *P. candelabraria* than *P. rauschi*. Cavities appear in some of the bends of the paruterine organ, although the cavity next to the uterus and a second near the anterior end become more conspicuous. Simultaneous with this internal development, the musculature in the wall of the proglottis appears to form a sphincter-like constriction, usually just anterior to the uterus. Development beyond this point was not observed in proglottides attached to a strobila.

Most of the shed proglottides had separated along the interproglottidal line, and further development had occurred. In some the eggs were still confined to the uterus, and the constriction in the body wall was pronounced, probably preventing movement of the eggs out of the uterus into the paruterine organ. The uterus projected beyond the posterior margin of the proglottis. The paruterine organ had developed also, now consisting of two pronounced

subspherical structures with relatively thick walls. One was immediately anterior to the uterus being separated from it by the constriction in the proglottis wall. The other sac was at the anterior end of the proglottis, frequently projecting beyond the anterior border. Connecting the two sacs of the paruterine organ was the sinuous structure, presumably hollow, which varied in the extent of dilation along its length in some proglottides but in others was fairly uniform in diameter.

In other proglottides, which were shed, most of the eggs were confined to the anterior sac of the paruterine organ. This thick-walled sac was greatly dilated and protruded almost in its entirety from the anterior end of the proglottis. A few eggs remained in the deflated uterus. The constriction in the wall of the proglottis was no longer noticeable.

Ripe proglottides in physiological saline were kept in the refrigerator in some cases for a month or more. The body wall decomposed, but the paruterine organ remained intact although light probing with a needle liberated the eggs. It appears that the paruterine organ gives the eggs more protection and keeps them together longer than if the eggs remain in the thin-walled uterus.

The Life History of *Paruterina rauschi* n.sp.

All stages of development of *P. rauschi* were obtained for study from controlled feedings. Eggs from ripe proglottides taken from a naturally infected barred owl were fed to eight house mice and a red squirrel (*Tamiasciurus hudsonicus*), and all became infected. A great horned owl was fed two mice with plerocercoids *in situ*. Mature *P. rauschi* were present when the owl was killed 47 days after feeding, although none of the worms contained eggs. Eggs from a second naturally infected barred owl were fed to five house mice and all were infected when examined later.

The Egg

The unfixed eggs measured from 35 by 20 μ to 41 by 22 μ (Fig. 6), and fixed eggs measured from 25 by 19 μ to 27 by 20 μ. The outer shell is thin and hyaline. The second envelope nearly fills the hyaline shell and contains within it a jelly-like substance in which occur hyaline globules of varying sizes. The embryo, within the second envelope, measured from 22 by 16 μ to 30 by 17 μ when fresh, but from 22 by 12 μ to 24 by 15 μ in fixed eggs. Three pair of identical embryonic hooks, from 8 to 10 μ in length, were on one of the long sides of the embryos.

Development of the Plerocercoid

Infected mice were killed at irregular intervals between 8 and 672 days after eggs were fed. The first mouse had small necrotic foci on the liver, primarily along the periphery, but no signs of infection in the pancreas or mesenteric lymph nodes. The liver lesions were examined in a squash preparation under cover glass, and developing plerocercoids were found in

some of the foci. The plerocercoids lacked a lacuna and varied from 640 μ in length and 320 μ in width to 980 by 580 μ (Fig. 7). The cuticle was much thicker on one end of the larva. Embryonic hooks were not seen.

Fourteen days after infection the most immature plerocercoid, measuring 866 μ by 460 μ, had a solid parenchyma with few calcareous granules confined to two longitudinal rows, one on each side. After standing for a time a part of an excretory duct became visible near one row of calcareous granules, but there was no sign of an excretory atrium. The scolex cone was less opaque than the remainder of the body and contained a scolex canal. The canal had a double dilation on the inner end. Presumably the innermost dilation would produce the rostellum, and the suckers would develop in the lateral walls of the upper dilation. There was no sign of hook development.

Other plerocercoids were almost fully developed after 14 days and measured up to 1.06 by 0.58 mm. They had a definite excretory atrium and pore on the non-scolex end of the larva, numerous calcareous granules, and the scolex, except for the rostellar hooks, was almost fully developed. The large rostellar hooks measured 36 to 37 μ in length and the small ones about 33 μ. Apparently the blade and part of the handle and guard grow from the same locus. The bulb of the guard appears to begin growth independently and on these hooks was shaped like a horseshoe. The bulb on the tip of the handle of these hooks was not yet formed and the handle end of the hook appeared to be open. The walls of the hook were thinner than on a fully developed hook.

One plerocercoid after 15 days of development had the developing scolex partly everted (Fig. 8). Primordia of the suckers and rostellum were visible, but hooks were absent. Fragments of excretory tubules were present, but there was no sign of an excretory pore. A few calcareous granules were scattered throughout the parenchyma. The cuticle was thickened, bluish-green in color, and full of bubbles, which appeared to emanate from the granular layer immediately under the basement membrane. The plerocercoid was somewhat flattened under the cover glass and just before rupture measured 1.65 by 0.87 mm. in maximum dimensions.

FIG. 6. Photomicrograph of live eggs of *P. rauschi* n.sp.

FIG. 7. Photomicrograph of live developing plerocercoid of *P. rauschi* n.sp.; note absence of lacuna in the plerocercoid 8 days old.

FIG. 8. Photomicrograph of anterior end of live developing plerocercoid of *P. rauschi* n.sp.; plerocercoid 15 days old, with primordium of suckers and rostellar pad, but no sign of rostellar hooks.

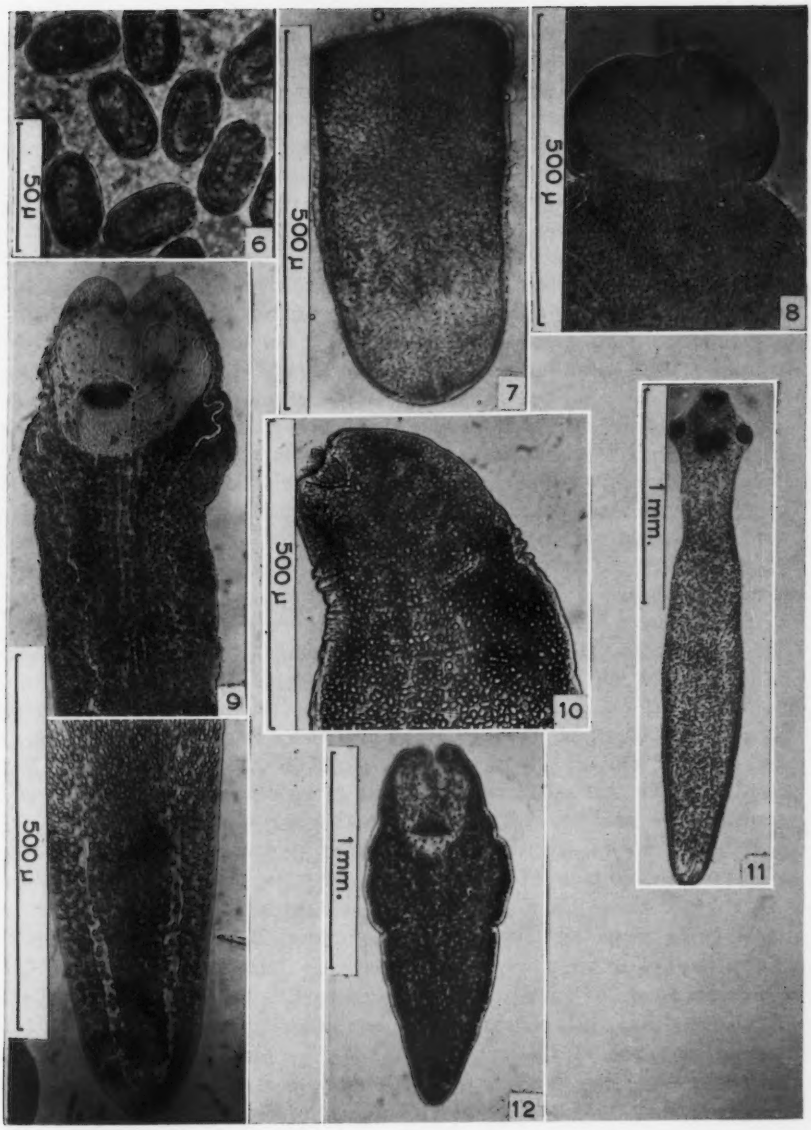
FIG. 9. Photomicrographs of anterior and posterior ends of a live fully developed plerocercoid of *P. rauschi* n.sp.; note scolex fully invaginated, also that dorsal and ventral excretory vessels are plainly seen with latter superficially appearing to connect directly to excretory atrium (see FIG. 5).

FIG. 10. Photomicrograph of anterior end of a live developing plerocercoid of *P. candelabraria* 26 days old; note sucker-like invagination which constitutes the scolex primordium.

FIG. 11. Photomicrograph of a live fully developed plerocercoid of *P. candelabraria*, 92 days old, with scolex everted, and body fully extended.

FIG. 12. Photomicrograph of a live fully developed plerocercoid of *P. candelabraria*, 26 days old, with scolex invaginated and body partly contracted.

PLATE I



Freeman—Can. J. Zool.

Other plerocercoids after 15 days of development measured from approximately 1.20 by 0.27 mm. to 1.05 by 0.20 mm. when fully expanded but without pressure of a cover glass. Many larvae everted the scolex spontaneously, although it was not fully developed in any of them. The degree of development of the rostellar hooks varied from plerocercoids with no hooks to those with hooks about as advanced as described from the mouse killed a day earlier.

After 17 days of development most plerocercoids were almost fully developed, but rostellar hooks still lacked a complete bulb on the guard and tip of the handle. The large hooks measured from 37 to 40 μ in length, and the small hooks were commensurate in development. Some plerocercoids were fully developed within 20 days, and after 23 days all plerocercoids were fully developed. Possibly some hooks increased further in length by additional growth at the tip of the handle after 23 days, but most hooks appeared to be fully developed after that time. Some plerocercoids were alive and normal after 672 days in one case. The plerocercoids were highly mobile and contracted and extended themselves. One plerocercoid when fully contracted was 0.8 by 0.47 mm. but measured 1.49 by 0.47 mm. when extended. One of the largest plerocercoids from a house mouse measured 2.1 by 0.46 mm., whereas from the red squirrel one plerocercoid was at least 2.9 mm. in length but contracted to about 1.4 mm. in length when covered with a cover glass.

The measurements in the following description of the fully developed plerocercoid are from 15 fixed, stained, and mounted specimens from seven experimentally infected house mice. Plerocercoids from the experimentally infected red squirrel were not significantly different. The plerocercoids varied from 0.57 by 0.27 mm. to 1.9 by 0.33 mm. (av. 0.99 by 0.26 mm.) in maximum dimensions. The everted scolex varied from 132 by 208 μ to 185 by 249 μ (av. 170 by 233 μ); the scolex cone, distance from anterior tip to posteriormost part of the invaginated scolex, from 214 to 249 μ (av. 228 μ) in length; suckers from 69 by 69 μ to 94 by 89 μ (av. 92 by 64 μ); rostellar pad 57 to 104 μ in width (av. 78 μ); and the rostellar ring 85 to 115 μ (av. 96 μ) in diameter. There were 41 to 49 rostellar hooks (av. 45); the large hooks varied from 36 to 43 μ (av. 41 μ) in length (the handle on some of these hooks may not have been fully developed), and the small hooks 31 to 34 μ (av. 33 μ) in length. The excretory atrium varied from 32 by 14 μ to 62 by 21 μ (av. 48 by 18 μ). The general form is as in Fig. 9.

The excretory system is particularly distinctive, and was studied from living and sectioned material. Towards each lateral margin, and extending posteriorly from within the scolex to near the posterior end of the plerocercoid, is a pair of excretory ducts. As in the mature strobila there is a larger duct, presumably the ventral, and a smaller dorsal duct. At the posterior end the ventral and dorsal ducts from each side turn mediad and unite with the respective partner from the other side (Figs. 5 and 9). The posterior transverse ducts lie immediately anterior to an excretory atrium which opens at the posterior tip of the plerocercoid. The excretory atrium is lined with cuticle which is continuous with the outer surface of the body. The inner end

of the atrium is ridged or has papillae. A tubule with a sphincter opens on each side from the larger excretory duct into the atrium, but no opening into the excretory atrium from the smaller duct was detected (Fig. 5). The smaller ducts are interconnected with the larger ducts in the scolex, however, which forms a complete system. Flame cells were seen throughout the length of the plerocercoid, and one plerocercoid was estimated to have 160 of them. The tubules from the flame cells could be traced only a short distance and no collecting tubules were observed to open into the longitudinal excretory ducts. Generally, the embryonic hooks were embedded next to the excretory ducts deep within the outer wall of the plerocercoid near the excretory atrium.

Certain detail was observed by allowing the cover glass to slowly flatten a living plerocercoid as the wet mount dried. Frequently the excretory atrium prolapsed and one could see that at least some of the papillae on the inner end were permanent, rather than temporary. The outermost layer of the cuticle was hyaline, and apparently semisolid, since as the plerocercoid died bubbles would form in the cuticle and burst to the outside. This hyaline layer was about 4 μ in thickness and contiguous with an inner granular layer approximately 2 μ in thickness. These two layers appeared to constitute the cuticle proper and were separated from the deeper tissue by a basement membrane. The cortical parenchyma, between the outermost layer of calcareous corpuscles and the basement membrane, was about 7 μ in thickness and was alveolar in appearance. Cellular detail was difficult to make out in this type of preparation.

P. rauschi plerocercoids readily everted the scolex when they were removed from host cysts, and occasionally several eversions and retractions of the same scolex were observed. In older infections, the scolex frequently was already everted when the larva was liberated from the host cyst; this occurred only rarely with *P. candelabraria*.

The eversion of the scolex permitted study of the active scolex, particularly the relationship of the rostellar hooks to the remainder of the scolex. On a "normal" retracted or everted scolex the hooks appear to rest directly on the rostellar pad, but when the scolex is fully distended and the rostellum pushed forward, the hooks can be seen resting on a ring of tissue which is above the rostellar pad. This tissue must be muscular since the hooks can act independently of the rostellar pad as well as in conjunction with it. The degree of eversion of the rostellum varied while it was invaginated within the scolex as well. On some invaginated rostella the hooks pointed anteriorly (Fig. 12), in others posteriorly (Fig. 9), and in some laterally, although the remainder of the scolex remained invaginated.

Natural Larval Infections

Three natural infections with *P. rauschi* plerocercoids were found in chipmunks from Algonquin Park. The measurements and morphology of the plerocercoids agree with the description already given. The first animal had only four plerocercoids in the liver, the second had several plerocercoids in the

same organ, and the third was heavily infected throughout the liver and mesenteric lymph nodes. Some of these plerocercoids were fed to captive owls as will be described below.

Hall *et al.* (7) reported finding plerocercoids of *Paruterina* sp. from the liver of *Peromyscus leucopus* in Maryland and Kentucky, U.S.A.; they did not describe or figure the plerocercoid in their paper. However, they kindly allowed me to examine sketches and measurements which they made of these plerocercoids and it is clear, particularly from hook morphology and measurements, that they had *P. rauschi.*

Mr. E. L. Schiller, of Johns Hopkins University, allowed me to examine a series of well-stained tapeworm plerocercoids which he recovered from the liver of a *Peromyscus leucopus* collected at the Patuxent Wildlife Refuge in Maryland, U.S.A. They were larger on the average* (from 1.41 by 0.23 mm. to 3.17 by 0.40 mm. in over-all dimensions) than any other plerocercoids encountered during this study. The hooks agreed in morphology with the hooks of *P. rauschi* as described above. The remaining measurements of the scolex, suckers, rostellar pad, and rostellar ring overlap or are larger than measurements of both *P. rauschi* and *P. candelabraria* (described below) on Ontario specimens. The morphology and measurements of the rostellar hooks clearly indicate that these larvae are *P. rauschi*, but the great size of the plerocercoids suggests that the apparent difference in size between the two species in specimens from Ontario may not be constant. There is a possibility that the specimens were overrelaxed before fixation, which contributed to this marked size difference. It is unlikely that age of the plerocercoids was directly involved, however, since in experimental infections plerocercoids about 10 weeks of age were not smaller than other larvae which were more than twice their age.

Development of the Adult

Plerocercoids of *P. rauschi* were fed to owls on three occasions. In the first feeding two of four plerocercoids from a natural infection in a chipmunk were fed to a juvenile eastern screech owl, but no cestodes were present 75 days later when the owl was killed. In the second instance a large number of plerocercoids, *in situ* in a naturally infected chipmunk, were fed to a juvenile long-eared owl, but the owl was not infected when killed 57 days later.

The third feeding resulted in infection after plerocercoids, while still *in situ* in two experimentally infected house mice, were fed to one of two juvenile great horned owls. The experimental owl was killed 57 days after the plerocercoids had been fed to it, but the uninfected control was not killed until another 77 days had elapsed. There was five strobilae in the posterior half of the gut of the infected bird, but, although most strobilae were sexually mature, no ripe proglottides were present.

*Seven plerocercoids were measured including the largest and smallest of the 18 which were present on the slides.

The Life History of *Paruterina candelabraria*

A complete life cycle of *P. candelabraria* was followed particularly to ascertain the morphology and rate of development of the larvae. Ripe proglottides from a naturally infected snowy owl were fed to various rodents, then some of these infected rodents were fed to a great horned owl, which in turn became infected and provided eggs with which other rodents were infected. Data on the feedings to rodents and rabbits are presented in Table II. Nine species of animals were exposed to eggs, but only house mice, two chipmunks (*Tamias striatus*), a white-footed mouse (*Peromyscus maniculatus*), and a red-backed mouse (*Clethrionomys gapperi*) became infected (Table II). It is unlikely that there were any natural infections among the hosts not reared in the laboratory, since *P. candelabraria* has never been found in any of the 2000 or so small animals examined from Ontario or Minnesota.

The Egg

The eggs, which measured up to 40 by 28 μ when unfixed, but only 33 by 21 μ to 35 by 24 μ when fixed, are somewhat larger than those of *P. rauschi*. The ovoid hexacanth embryo, in fixed material, measured from 25 by 13 μ to 27 by 18 μ; the identical embryonic hooks were 9 to 10 μ in length.

Development of the Plerocercoid

An infected house mouse showed small white diffuse necrotic areas on the liver eight days after eggs had been fed to it, the earliest time that an infection with *P. candelabraria* was detected. These spots were removed and examined in squash preparations. In two of them developing plerocercoids were found, unencysted, although surrounded by necrotic tissue. These plerocercoids were elongate, 350 by 170 μ and 175 by 115 μ in dimensions respectively. Both plerocercoids still had the six embryonic hooks. On the smaller worm the hooks were distributed along the length of the middle third of the worm. The larger one had a few calcareous corpuscles and the embryonic hooks were more toward one end. There was no lacuna in either plerocercoid. There was a pronounced cuticle, and muscle fibers probably were present, because the body expanded and contracted, but other cellular differentiation was not observed.

The next infected house mouse was examined 26 days after eggs had been fed to it. There were eight plerocercoids in individual foci along the periphery of the liver lobes. All had a solid parenchyma throughout the length of the worm. Six plerocercoids had fully developed scoleces. Superficially the other two resembled the fully developed specimens, being elongate, 1.75 mm. in length and 0.4 mm. in maximum width, with the anterior end broadly rounded and a more pointed posterior end, but there was a marked difference in the degree of development of the scolex. The only sign of a scolex was a sucker-like invagination which had outside dimensions approximately 80 μ deep and 130 μ wide (Fig. 10). In every other respect the plerocercoid appeared fully developed as will be described below.

TABLE II

RESULTS OF FEEDING EGGS OF *Paruterina candelabraria* TO VARIOUS MAMMALS

Host species	Source of eggs	How fed	Number fed	Number infected	Range of time in days when hosts killed	Organs infected
Sciuridae						
Tamias striatus	Snowy owl	Food pellet	3	1	7–179	Liver and mesenteric lymph nodes
Tamias striatus	Great horned owl	Food pellet	1	1	92	Liver and mesenteric lymph nodes
Sciurus carolinensis	Snowy owl	On lettuce	2	0	109–213	—
Tamiasciurus hudsonicus	Great horned owl	Food pellet	1	0	83	—
Cricetidae						
Clethrionomys gapperi	"	Food pellet	2	1	72– 83	Mesenteric lymph nodes
Peromyscus maniculatus	Snowy owl	Food pellet	1	1	149	Liver, pancreas, and mesenteric lymph nodes
Muridae						
*Mus musculus**	"	Stomach tube	15	13	8–311	Liver and mesenteric lymph nodes
*Mus musculus**	Great horned owl	Food pellet	9	1	15– 49	Liver
Erethizontidae						
Erethizon dorsatum	Snowy owl	Stomach tube	1	0	74	—
Caviidae						
*Cavia porcellus**	"	On lettuce	2	0	96–133	—
Leporidae						
*Oryctolagus cuniculus**	"	On lettuce, stomach tube	2	0	73–189	—

*Hosts reared in the laboratory.

Many fully developed plerocercoids, varying from 26 to 311 days of age, from the mesenteric lymph nodes and liver of four species of hosts were studied. The size of plerocercoids is similar in both organs and in the different hosts. Living plerocercoids showed most details best, but they were difficult to measure owing to movement. Some everted the scolex on standing in physiological saline, although this occurred more often with *P. rauschi.*

Fully developed living plerocercoids varied from 1.02 by 0.20 mm. to 2.85 by 0.33 mm. (av. 1.93 by 0.41 mm.)* in maximum over-all size in contrast to fixed specimens which varied from 1.03 by 0.30 mm. to 2.06 by 0.27 mm. (av. 1.47 by 0.37 mm.) (Figs. 11 and 12). All other measurements given here are from fixed and stained preparations. The everted scolex varied from 180 by 250 μ to 200 by 350 μ (av. 190 by 280 μ); scolex cone from 250 to 390 μ (av. 300 μ) in length; suckers from 80 by 60 μ to 110 by 100 μ (av. 100 by 70 μ); rostellar pad from 50 μ in length by 90 μ in width to 40 by 130 μ (av. 50 by 100 μ); rostellar ring from 110 to 150 μ (av. 125 μ). There were from 39 to 46 (av. 42) rostellar hooks, the longer ones from 49 to 56 μ (av. 53 μ) and the shorter ones from 36 to 42 μ (av. 39 μ) in length. Calcareous granules were scattered throughout the length of the plerocercoid but were less numerous towards the extremities. These ovoid and irregularly shaped granules varied from 13 by 8 μ to 24 by 14 μ (av. 16 by 12 μ). The excretory system was essentially the same as described for *P. rauschi*; the excretory atrium varied from 42 μ in depth by 11 μ in width to 83 by 25 μ (av. 56 by 19 μ), and looked smaller in respect to the rest of body than in *P. rauschi.* Plerocercoids of *P. candelabraria* were on the average twice as large as those of *P. rauschi,* and the plerocercoids of the two species were separated readily on the morphology of the hooks.

Development of the Adult

A great horned owl, more than a year old, was fed three house mice and a chipmunk each with fully developed plerocercoids *in situ.* The owl contained over 25 strobilae, and strobilar fragments, tightly bunched in the posterior third of the small intestine, when killed and examined 45 days later. Some egg-containing proglottides, which occurred in the large intestine, were used to infect mice. This is in contrast with *P. rauschi* in a great horned owl, which had no egg-bearing proglottides 57 days after exposure. The latter host was a juvenile bird, whereas *P. candelabraria* were fed to an adult. Therefore, if the assumption is correct that young of a species are more readily infected than older animals, adult great horned owls may be even less satisfactory hosts for *P. rauschi.*

Host–Plerocercoid Relationship

Some observations were made on the reaction of the host to the presence of the plerocercoid, most observations being the same for both species of worm. The lesions in the liver varied from diffuse whitish areas of necrosis to small

*In most cases 10 or more measurements were used to compute the average.

discrete host cysts. Lesions were more common along the periphery of the liver lobes, but the distribution varied from host to host. Generally in lighter infections the peripheral distribution was more pronounced. The earliest lesions always were diffuse areas, but frequently the plerocercoid occurred outside this area. As late as 17 days after infection, plerocercoids were seen free in the liver tissue of one host, although in other hosts after only 8 days the larvae were confined to a lesion and host cyst formation had begun. One host 15 days after infection had clear, hyaline host cysts in which plerocercoids, which appeared milky white, were confined. Most commonly, however, the lesions whether diffuse or compact were whitish and opaque. In some hosts the reaction was extensive and yet no worms occurred in some lesions, suggesting the possibility that other microorganisms were present. In some older infections (94 and 672 days) there was pronounced black pigment along the periphery of the host cyst. Plerocercoids up to 3 months of age were liberated readily from most host cysts in the liver of infections, but as the cysts grew older they became hard and slippery and were difficult to open. Such cysts varied from 0.54 to 1.6 mm. in maximum dimensions. The host cysts, especially in older infections, contained "debris" composed of crystalline material, host macrophages, and cellular fragments in addition to the plerocercoids. Some plerocercoids were still alive after 672 days, although others in much younger infections (one as early as 25 days) were overcome. Many plerocercoids from liver cysts in older infections (after about 150 days) were lethargic or totally inactive when released from the host cyst, although completely normal in appearance. It is questionable if the inactive ones were infective, although some had active flame cells.

Host cysts in the mesenteric lymph nodes, especially in older infections, were slightly discolored and the whole organ presented a turgid lumpy appearance; positive detection of infection required shredding of the organ. The lesions in the mesenteric lymph nodes differed from those in the liver in several ways. Many larvae were found in each cyst, while in the liver more than one or two plerocercoids per cyst was rare. The cysts in the mesenteric lymph node usually were large and appeared honey-combed when opened. Occasionally larvae could be seen when the cysts were *in situ*, but usually the cysts were opaque. A hard host cyst was never encountered in the mesenteric lymph nodes, and most if not all larvae were active, even in the oldest infections.

The liver was most commonly infected, and simultaneous infection of liver and mesenteric lymph nodes also was common, but only rarely were the mesenteric lymph nodes the only organ infected. An exact count was not made, but it is very likely that there were as many larvae, if not more, removed from the mesenteric lymph nodes as from the liver. The pancreas was infected only once.

Limited evidence suggests that younger mice are more readily infected than older ones, but a critical study was not made. Limited evidence also

indicates that hosts already harboring one species of larval cestode are difficult to infect with *Paruterina* spp. There was no evidence that the larvae grossly affected the vitality of the host.

Host Specificity and Geographical Distribution

In Ontario *P. candelabraria* was found in two of four snowy owls and an experimentally infected great horned owl. In addition specimens of *P. candelabraria* were identified from great horned owls from the north central United States and Montana, from a long-eared owl from Montana, from a snowy owl from North Dakota, and from a saw-whet owl in Quebec (Table I). *P. rauschi* occurred in two barred owls examined in Ontario, and a great horned owl was infected experimentally. Specimens of *P. rauschi* were examined from a barred owl from Maryland and Iowa in the United States. Four larvae of *P. rauschi* from an Ontario rodent failed to infect a screech owl (*Otus asio*) when fed to it, and similar larvae failed to infect a long-eared owl. No tapeworms occurred in the following from Ontario: seven great horned owls, one screech owl, one barn owl (*Tyto alba*), and two long-eared owls.

Rausch (16, 17) examined at least 87 owls of nine species and found *Paruterina* in eight great horned owls and two or possibly three barred owls. He could not identify with certainty some decomposed cestodes from two screech owls or immature cestodes from a snowy owl. One of his specimens from a great horned owl (No. 46327, U.S. National Museum), already mentioned above, is *P. candelabraria*, and it is reasonably certain that at least two of the barred owls contained *P. rauschi*.

The above evidence suggests that *P. rauschi* is primarily a parasite of the barred owl and occurs in the south-temperate zone of North America. It can infect the great horned owl. *P. candelabraria*, on the other hand, occurs primarily in owls of north-temperate and arctic zones. It may be primarily a parasite of the far north, occurring in the snowy owl, but it can and does infect great horned, saw-whet, and long-eared owls. Thus far all specimens of *P. candelabraria* in the latter three species have occurred where their range overlaps the southern limit of distribution of the snowy owl. Apparently tapeworms are more common in owls from North America than from Europe.

The distribution of *P. rauschi* plerocercoids further supports some of the above conclusions. Plerocercoids of this species have been found three times in approximately 2000 small animals which were examined in Ontario, and plerocercoids of the same species have been identified from rodents in two areas of Maryland, and from Kentucky. Natural infections with plerocercoids of *P. candelabraria* have not been found.

The limited evidence suggests that *P. rauschi* adults may be more host specific than *P. candelabraria*. As might be expected the plerocercoids are not very host specific, however, since those of the latter species will infect various species of at least three families of smaller rodents (Table II), and the former readily infected house mice and a red squirrel.

Discussion

The immature stage of *Paruterina* differs from that of most other cyclophyllideans. The two common types encountered in this order are the cysticercus (or variant of it), and the cysticercoid (8, 23). Both of these forms have a lacuna, the "primitivhühle" of German authors, in their development; the cysticercus retains this lacuna as the cavity of the bladder, whereas it frequently largely disappears in the mature cysticercoid. The cysticercoid usually possesses a tail-like appendage, at least at some time in its development, and generally the embryonic hooks are found embedded in its wall. The hooks are lost when and if the appendage is lost. The cysticercus lacks a tail-like appendage, and the embryonic hooks are difficult to see after early development of a cysticercus, at least in species like *Taenia mustelae* and *T. crassiceps*, although Benham (3) indicates they are present in the bladder wall of *Taenia* sp. Characteristically, the cysticercoid scolex is retracted but, unlike a cysticercus, it is not invaginated within itself (8, 23). The retraction of the scolex in cysticercoids produces a double-layered bladder wall, whereas the cysticercus has only a single-layered bladder wall.

It is evident that immature *Paruterina* are neither cysticerci nor cysticercoids. They are, however, similar in basic morphology to the tetrathyridia of species of *Mesocestoides*, another cyclophyllidean genus, differing primarily in the possession of an armed rostellum. Baer (1) referred to a larva of similar morphology, which he believed to be the larva of *Taenia polyacantha*, as an armed tetrathyridium. Later, Joyeux and Baer (10) called the larva a plerocercoid;* they feel that tetrathyridium is a specialized name for this cyclophyllidean plerocercoid just as sparganum is used for certain pseudophyllidean plerocercoids. Wardle and McLeod (23) also consider tetrathyridia and plerocercoids as identical and state: ". . . a plerocercoid is a *solid* larval tapeworm in which the future holdfast is present as an invaginated structure, but from which the embryonic hooks have disappeared. The stage begins with the shedding of the cercomer—or loss of the hooks—and it ends with the beginning of proglottisation." The tetrathyridia of *Mesocestoides* lack embryonic hooks, probably losing them during an earlier stage of development as occurs in the procercoid of Pseudophyllidea. (Most recent workers postulate a first intermediate host in the life cycle of *Mesocestoides*, but the existence of one has not been demonstrated conclusively.) Thus, morphologically, the immature stage of *Paruterina* is a plerocercoid, although it neither develops a cercomere nor sheds the embryonic hooks, and lacks a separate developmental stage comparable to the procercoid.

Paruterina and *Mesocestoides* are not the only genera of Cyclophyllidea with plerocercoids. The immature stages of *Oochoristica ratti* (18) which develop in beetles, and *Cladotaenia* spp. (personal observations), which develop in mice, are plerocercoids which retain the embryonic hooks. *Cylindrotaenia americana* (9) and *Catenotaenia pusilla* (11) have plerocercoids also. It is

*Schiller (21) has shown that immature *Taenia polyacantha* are typical cysticerci, rather than solid-bodied larvae as described by Baer.

not clear from the figures of *C. americana* whether this stage retains the embryonic hooks, but they are lost early in the development of *C. pusilla*. The life cycles of the latter two species are of particular interest because the plerocercoid is a transitional stage, which occurs in the definitive host. Joyeux (9) believes that there is no intermediate host in the life cycle of *Cylindrotaenia*. *Catenotaenia* develops in a tyroglyphoid mite into the unique "merocercoid", which is a solid-bodied stage with an apical sucker, two protonephridia, and a small terminal excretory vesicle. The apical sucker may be homologous with the rostellum of many cyclophyllideans, but is resorbed when the typical scolex develops in the definitive host. All these observations suggest that the cyclophyllidean life cycle with a plerocercoid is more common than was once suspected.

There is speculation whether the ancestral immature cyclophyllidean was a primitive cysticercoid, cysticercus, or coenurus (4). However, if the Cyclophyllidea arose from Tetraphyllidea, which have a plerocercoid stage (2, 23), then it follows that Cyclophyllidea with a plerocercoid are the older genera in their order. Further, the plerocercoid is solid-bodied and has a terminal excretory pore, whereas both the cysticercus and cysticercoid have a lacuna and an excretory pore which develops at the base of the scolex rather than being terminal (personal observations; 2). Apparently the new location of the excretory pore and development of the lacuna are recently acquired modifications among the higher Cyclophyllidea; thus, it is most likely that both cysticerci and cysticercoids arose from a solid-bodied plerocercoid more or less simultaneously rather than in sequence.

The immature stage of *Oochoristica deserti* Millemann, 1956 is of considerable interest. Unlike *O. ratti*, which lacks a lacuna and develops into a plerocercoid, *O. deserti* has a lacuna which remains until the immature stage is fully developed (15). The scolex begins development while everted, which is characteristic of a cysticercoid rather than a cysticercus, but when the scolex is fully developed it invaginates, rather than retracts, creating a compact cysticercus with a single-layered bladder wall. Before invagination of the scolex the larva has a small terminal invagination on the non-scolex pole, which resembles the excretory atrium on a plerocercoid, but Millemann never saw excretory ducts (15). Truly, this immature form is intermediate between the plerocercoid on the one hand and the cysticercus on the other.

The development of *Dipylidium caninum*, based on Venard's (22) thorough study, is equally interesting. In the flea larva and pupa a lacuna develops in the onchosphere, but when the adult flea emerges the lacuna fills up with parenchymatous tissue before other differentiation occurs. Then a tail appendage, or cercomere, with the embryonic hooks is formed, and development of the rostellum, followed closely by that of the suckers, begins. Apparently the latter two structures invaginate as they develop, so that the scolex is invaginated when fully developed. The cercomere drops off at some time in development, although Venard does not say when, and the excretory system such as already described for plerocercoids develops. Thus when fully

developed this is a plerocercoid (see 22, Fig. 45). This type of development, at best, is a precursor to that of the typical cysticercoid.

The present-day taxonomic arrangement of the order Cyclophyllidea is based exclusively on adult morphology (23). The genus *Cladotaenia*, which produces plerocercoids, along with the genus *Taenia*, which produces cysticerci, are placed in the family Taeniidae. Some workers still place the genera *Oochoristica*, with plerocercoids and cysticerci, and *Catenotaenia*, with plerocercoids, in the family Anoplocephalidae, along with *Moniezia*, *Monoecocestus*, etc., which produce cysticercoids. The genus *Paruterina*, with plerocercoids, and the genus *Metroliasthes*, which produces cysticercoids, are placed in the same family. Undoubtedly, other similar anomalous arrangements exist. Clearly, the diversity in the development and morphology of immature stages of the cyclophyllidean cestodes mentioned above should be considered in any future taxonomic revision if it is intended to clarify natural relationships.

Acknowledgments

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RESPONSES OF JUVENILE CHUM, PINK, AND COHO SALMON TO SHARP SEA-WATER GRADIENTS[1]

ARTHUR H. HOUSTON[2]

Abstract

The responses of chum, *Oncorhynchus keta* (Walbaum), and pink, *O. gorbuscha* (Walbaum), salmon fry, and coho, *O. kisutch* (Walbaum), salmon fry and smolts to sea water were studied in sharp-gradient tanks. Chum and pink fry responded positively to isotonic and hypertonic solutions of sea water, but coho fry only to the former. During parr–smolt transformation, coho responded positively to hypertonic sea water. Responses of chum fry acclimated to sea water for 24 hours prior to observation were comparable in intensity to those of unacclimated fry, but less rapid. Activity of acclimated fry decreased less rapidly than did that of unacclimated fry. Fresh water control experiments indicated the presence of some factor or factors which resulted in preferences for "recognized" areas. The effects of positive responses to increased salinity are discussed in relation to the migratory movement of these species from fresh water into the sea.

Introduction

The ability of teleost fishes to detect small differences in salinity is acute (3) but salinity gradients in the open ocean appear to be too irregular in pattern and insufficiently steep to provide reliable cues for navigation. The same situation apparently prevails in fresh-water systems and is enhanced by the low over-all concentration of dissolved solids (2, 4). However, salinity gradients may become potential directive factors in regions such as river mouths and estuaries where gradients are both marked and continuous in direction. Juvenile Pacific salmon, with their variable migratory behavior, provide valuable material for investigating the development of responses to increased salinity. Chum and pink salmon migrate seaward soon after hatching, while coho tend to remain in fresh water for at least one year, moving seaward after parr–smolt transformation. Differences in response to sea water might therefore be expected to be a part of the behavioral pattern of these three species, and it was the purpose of the present investigation to study this aspect of their ethology.

Material and Methods

Salmon used in these experiments together with pertinent data are listed in Table I. Stocks were maintained in the University laboratory and were fed a mash of canned salmon and cereal (Pablum) with strained liver added to their diet once weekly.

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Contribution from the Department of Zoology and the Institute of Fisheries, University of British Columbia, with financial assistance from the Research Council of Ontario and the National Research Council of Canada.
[2]Graduate Student.

Apparatus

Reactions were studied by offering the fish the choice of remaining in fresh water or moving into sea water. A sharp-gradient tank (Fig. 1) modified from the design used by Baggerman (1) was employed. Each tank measured 60 by 18 by 30 cm. deep and was divided into two compartments of equal volume by a central partition 23 cm. high. The tanks were painted throughout with a non-toxic gray enamel. A water 'bridge' 2 cm. high over the central partition enabled the fish to move between compartments. This was established, after the initial filling of the two compartments, by running fresh water into the fresh-water compartment to the desired height. During the operation some exchange of water could not be avoided. A typical salinity profile (Fig. 2) shows the distribution of sea water between compartments midway through the period of observation. The arrangement of lights and mirrors (Fig. 1) provided a means of illumination (1.17 foot-candles at the water surface) and observation. A surrounding bath of running water at the same temperature as that in the fish culture troughs maintained temperature within 2° C. Slight differences in oxygen concentration existed between compartments (11.2 ± 0.3 mg. oxygen/liter in fresh water, 9.4 ± 0.1 mg. oxygen/liter in sea water) but the results of the experiments suggest that they had little influence on the orientation of the fish.

Procedure

The same procedure was followed in all experiments. One compartment was filled with fresh water to the level of the partition and the alternate with test solution of sea water or, in the case of control experiments, with fresh water. In reporting results the former are referred to as "fresh-water compartments" and the latter as "sea-water compartments."

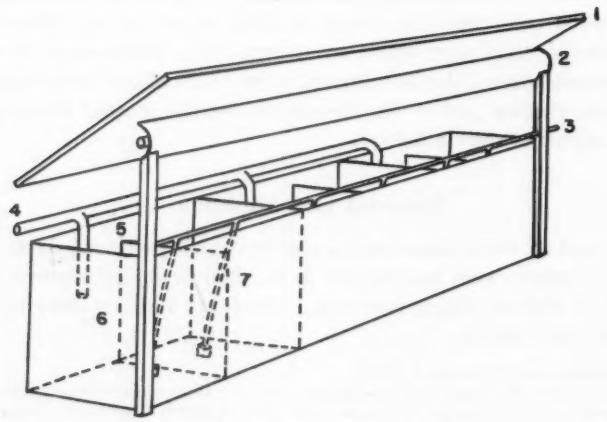


FIG. 1. Apparatus showing three pairs of gradient tanks: 1, mirror; 2, lighting system; 3, air supply lines; 4, fresh-water supply line; 5, partition; 6, "fresh-water compartment"; 7, "sea-water compartment".

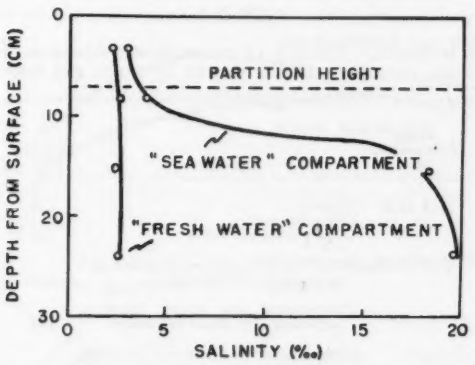


FIG. 2. Typical salinity profile through gradient tank midway through period of observation.

Groups of 8 to 12 fish were placed in the "fresh-water compartments" and left undisturbed for six to eight hours to recover from any shock of transfer. Following this, aerators were turned off to decrease water exchange and fresh water was slowly added to the "fresh-water compartments" to establish the 'bridge'. Every precaution was taken to avoid disturbance of the fish during this operation.

The number of fish in each compartment was determined at 2 minute intervals for 10 minute periods starting at 0, 10, 25, 45, 75, 105, 165, and 195 minutes after completion of the bridge. The percentage of fish in the "sea-water compartments" was taken as a measure of the response of the fish to the solution being tested.

MacKinnon and Brett (14) and Neave (15) have shown that maximum migratory movement occurs during the late evening, and reaches a peak near midnight. Accordingly the actual observations were carried out during this period.

Chi-square analysis was used to determine the uniformity of distribution of fish between compartments in control experiments. Following this the "2 by N" test (17) was applied to test for significant differences among the distributions (responses) of control and experimental groups of fish. In cases where some individual values of a particular experiment did not exceed the chosen level of significance (95%) a summation of values followed by their comparison against the appropriate chi-square value was used to test for over-all significance between distributions.

A measure of the stability of distribution and thus indirectly of the activity of the fish was made by determining the average number of exchanges between compartments per fish per minute. Values obtained in this way were admittedly subject to many variables but were useful indications of gross changes in the activity of fish under different experimental conditions.

Acclimation to sea water prior to observation was carried out in small, aerated aquaria held at culture trough temperatures. The salinities of acclimation and experimental solutions are presented in Table I.

TABLE I

EXPERIMENTAL MATERIAL. FIGURES IN PARENTHESES, STANDARD DEVIATION OF MEAN FORK LENGTH; SALINITY VALUES IN PARTS PER THOUSAND

Species	Origin	Mean fork length, mm.	Experiment	Sample size	Mean salinity	
					Test sol.	Acclimation sol.
Chum fry	Cultus Lake	38.7 (2.4)	Control	184		
			Dilute sea water	181	5.10	
			Concentrated sea water	182	19.43	
		42.9 (3.5)	Dilute sea water, following acclimation to concentrated sea water	138	6.02	22.22
			Concentrated sea water, following acclimation to dilute sea water	153	23.69	9.23
			Concentrated sea water, following acclimation to concentrated sea water	245	22.99	22.59
Pink fry	Oyster River	33.4 (1.7)	Control	107		
			Dilute sea water	140	8.27	
			Concentrated sea water	84	20.34	
Coho fry	Cultus Lake	34.7 (2.3)	Control	206		
			Dilute sea water	170	7.31	
			Concentrated sea water	180	23.96	
Coho smolt	Salmon River	57.4 (6.5)	Control	180		
			Concentrated sea water	182	21.83	

Results

CHUM SALMON FRY

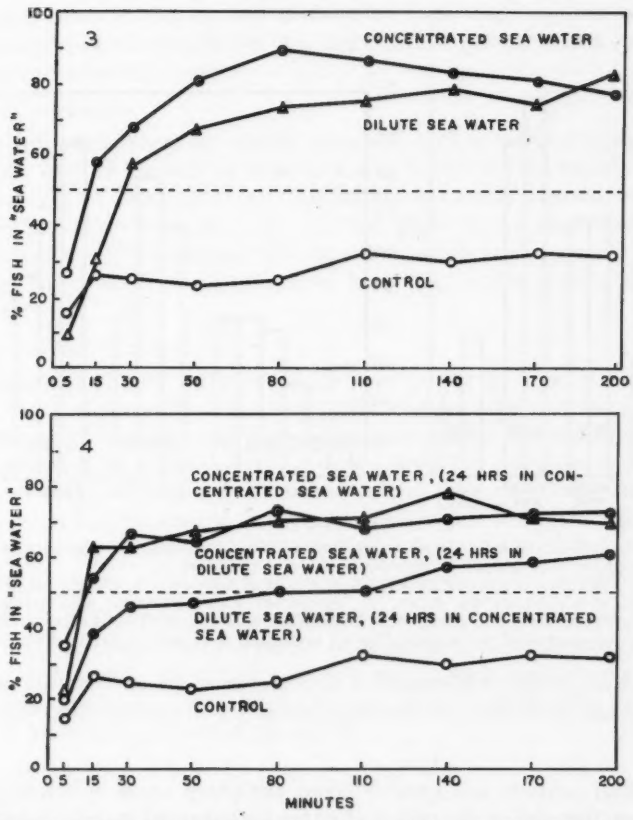
General Observations on Behavior

During the resting period fry swam actively through their compartments and were not concentrated in any particular area. Occasional orientation to currents set up by aerators was observed, and orientation to inflowing water was usual while the bridge was being established. In a few cases disturbance of the fish caused them to form loose, relatively inactive groups near the sides and bottom of the compartments.

On completion of the bridge the fry schooled and swam rapidly back and forth in the area above the partition with individuals occasionally diving into either compartment. During this phase of group behavior, which generally lasted for 30 to 50 minutes, the activity of the fry appeared to be at a maximum. Activity gradually decreased following the breaking up of the schools and the expression of individual rather than group responses by the fish.

Control Experiments

In control experiments fry choosing between their original compartments and alternate fresh-water compartments showed a marked tendency to remain in the former (Fig. 3). Chi-square values consistently exceeded the 99% level of significance, and this together with the high summation value of the statistics indicated small probability that the distribution had occurred by chance.



FIGS. 3 and 4. Responses to sea water of unacclimated chum fry (FIG. 3) and acclimated chum (FIG. 4); solid points, distribution significantly different from control at 95% level.

Response to Sea Water

In contrast to control fish, fry having the choice between their original fresh-water compartments and those containing dilute sea water showed a strong preference for the latter (Fig. 3). Response was both rapid and intense, with significant numbers of fry being concentrated in sea water within 30 to 50 minutes after observations were begun. An even more rapid reaction was exhibited to concentrated sea water (Fig. 3) although the final response was about the same in either case.

The effect of acclimation to sea water is shown in Fig. 4. Significant responses to both concentrations of sea water were still apparent, although in most cases they were initially less rapid than those displayed by fry entering sea water for the first time. If this behavior is typical of that shown by fry in their natural surroundings there is little likelihood that the fish would 'stray' back into fresh water following contact with sea water. It is interesting

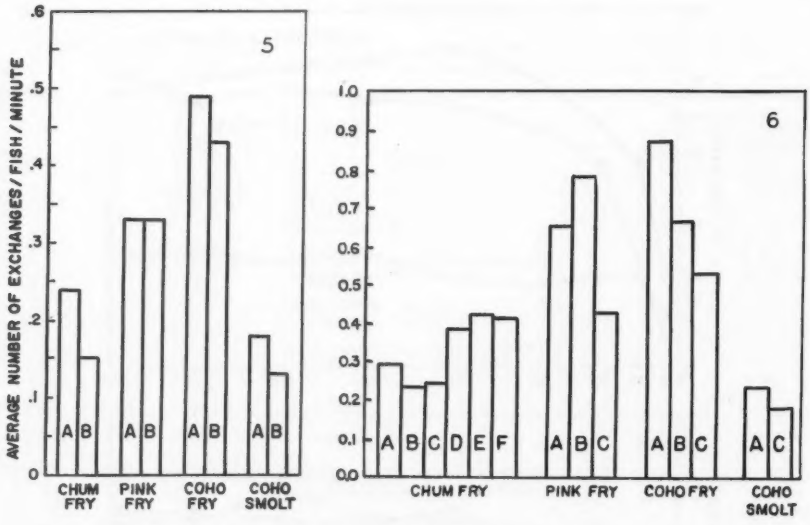


FIG. 5. Activity of fish in fresh water during individual and group phases of response; A, group phase of response; B, individual phase of response.

FIG. 6. Activity of fish during individual phases of response: A, control; B, dilute sea water; C, concentrated sea water; D, dilute sea water following acclimatization to concentrated sea water; E, concentrated sea water following acclimatization to dilute sea water; F, concentrated sea water following acclimatization to concentrated sea water.

to note that major differences in concentration of acclimation solutions (Table I) had little effect on the responses of fry to concentrated sea water.

Activity

In general, activity was greater during the group phase of behavior (30 to 50 minutes) than during the period when the fry appeared to react as individuals rather than as schools (Fig. 5). This decrease in activity was more noticeable in unacclimated fry than among fry in fresh water and those which had been acclimated to sea water (Fig. 6). These data suggest an immediate effect of sea water on the motor activity of fish entering sea water for the first time.

PINK SALMON FRY

General Observations on Behavior

The general behavior of pink fry was similar to that of chum fry with respect to orientation and grouping, but these fish appeared to be much more susceptible to disturbing stimuli than chums. Following the establishment of the bridge the fry schooled and swam vigorously in the areas above the partitions. Individuals dived frequently into either compartment and then rejoined the main school. Their activity was noticeably higher than that of chums and this high level of activity was maintained throughout the whole period of observation. Schooling behavior seemed to be more intense in

this species than in chums, and division of their reactions into group and individual preference phases was less apparent both from direct observation and with reference to activity

Control Experiments

In control experiments fry usually remained in their original compartments during the first 30 minutes of observation and then moved rapidly over the partitions (Fig. 7). Although their distribution was nearly uniform, summation of chi-square values indicated a tendency for the fry to remain in their original compartments. This tendency was, however, much less apparent than that displayed by chum fry and may have been due to the greater activity of the former.

Response to Sea Water

The response of pink fry to sea water was neither as rapid nor as marked as that of chum fry, although a fairly clear-cut positive reaction to concentrated sea water was recorded during the latter half of the experiments (Fig. 7). Summation of individual chi-square values for response to dilute sea water also indicated a positive reaction to this concentration. As was the case for chum fry, response to concentrated sea water was more marked than that to dilute sea water.

Activity

Unlike chum fry, pink salmon exhibited a relatively high and sustained level of activity throughout the period of observation in fresh water. As was the case with chums the activity of this species decreased after entry into concentrated sea water, although there was a slight increase after the fish had moved in the dilute solution of sea water.

Coho Salmon Fry

General Observations on Behavior

During the preliminary resting period coho fry were relatively inactive, remaining stationary on or near the bottom, or swimming slowly in the lower regions of their compartments. Orientation to aerator currents was less common than with chum and pink fry while nipping (7) was more prevalent. Establishment of the bridge appeared to disturb the fish in most cases and resulted in the formation of loose, inactive groups near the corners of the compartments. Orientation to inflowing water was uncommon. Following completion of the bridge the fry formed schools in the upper regions of the tanks. Those in fresh water (control) and those facing dilute sea water were observed to dive impartially into either compartment. Those facing concentrated sea water dived less frequently into sea water than into fresh water.

Control Experiments

The most noticeable feature of the distribution of control fry was its uniformity (Fig. 8). Except in one instance, observation at 170 minutes,

all chi-square values were below the 95% level of significance. In view of this and the low summation value of the individual chi-squares it seems apparent that the tendency to remain in their original compartments, which was manifested by chum and pink fry, was not characteristic of coho fry. This pattern of behavior may be associated with the strong territorial drive exhibited by this species, occupation and defence of individual territories tending to scatter the fish.

Response to Sea Water

The response of fry to dilute sea water was both rapid and intense, with a significant percentage of fish found in sea water at all times after 50 minutes. Since coho do not normally migrate seaward as fry, this response is at first consideration anomalous. It may, however, constitute a preference for a medium in which the least amount of osmotic work is required rather than a response to sea water as such.

The graphical presentation of the reaction of the fish to concentrated sea water (Fig. 8) is misleading in that it would seem to indicate no preference for either fresh or sea water. There was, however, a complicating factor. Fry recorded in sea water were observed to stay mainly in the upper few centimeters of the tank, a region already indicated (Fig. 2) to be dilute in concentration. A rough estimate of the vertical distribution of the fish showed that on the average 50 to 60% of the fry in sea water were to be found in this region, which made up less than one-third of the total volume of the tank. By contrast fry in fresh water and those in dilute sea water were uniformly distributed. Again this suggests a preference for dilute sea water over both fresh water and concentrated sea water. The response to concentrated sea water was, however, not positive.

Coho Salmon Smolts

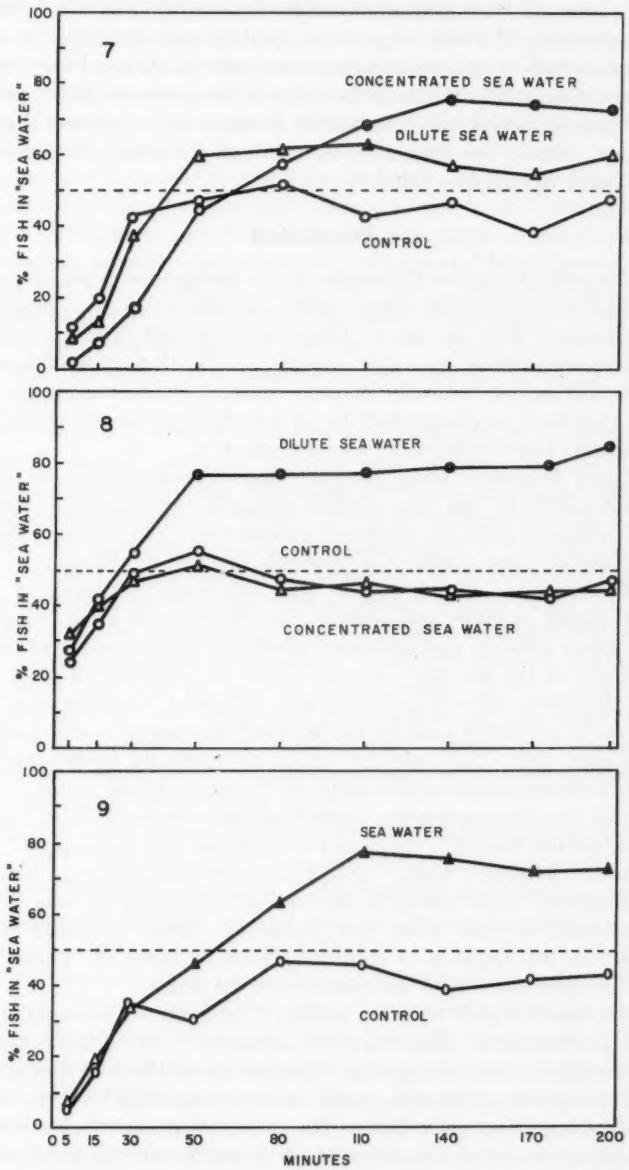
General Observations on Behavior

During the recovery period, the smolts swam actively throughout their compartments and there was little evidence of orientation to aerator currents. Nipping was prevalent, and in a few cases hierarchical systems (18) were observed. Following completion of the bridge the fish moved slowly over the partitions as individuals rather than in schools. Little nipping was noticed among fish which had entered sea water.

Control Experiments

The distribution of smolts in fresh water, while nearly uniform, had a bias towards the compartments in which the fish had originally been placed (Fig. 9). Summation of chi-square values over the last 150 minutes of observation gave a value exceeding the critical level for 95% significance. This suggested a reaction of coho smolts comparable to that seen in chum and pink fry under similar conditions. In this reaction and in their reactions to

sea water, coho smolts resembled chum and pink fry more than coho fry, a convergence in behavior patterns already described by Hoar (7, 9).



FIGS. 7–9. Responses to sea water of pink fry (FIG. 7), coho fry (FIG. 8), and coho smolts (FIG. 9); solid points, distribution significantly different from control at 95% level.

Response to Sea Water

Response to concentrated sea water was slow but positive. In this the behavior of the smolts was markedly different from that of fry. Comparison of the responses of the two stages to equivalent concentrations of sea water gave values which were consistently above the critical level for 95% significance, indicating a definite change in response to sea water with age. As for fry of all species, activity of coho smolts in sea water was lower than that in fresh water. Again this suggested an effect of sea water on the activity of fish entering it for the first time.

Discussion

The data indicate species differences in the occurrence of positive responses to sea water among juvenile chum, pink, and coho salmon. These responses can be correlated with the known behavior of the fish since marked positive reactions to *concentrated* sea water occurred only in that stage of development when the fish would normally migrate seaward. The correlation strongly suggests that such responses may be an integral part of the behavior which leads migrants from fresh water into the sea.

Rheotactic responses have generally been considered to be the major behavioral factor in the downstream migration of Pacific salmon (7, 9). River mouths and estuaries, however, present situations in which the orientational values of these reactions is probably decreased. Rheotactic reactions are dependent upon visual, tactile, and labyrinthine stimuli (6) and the increased depth of river and stream channels in such areas, together with reduced water velocity and decreased constancy of flow patterns, may bring the intensity of the necessary stimuli below the level for effective response. In such situations salinity gradients may assume a supplementary, if not a major role, in providing cues for the seaward movement of migrants.

It has been noted that coho fry which are normally fresh-water residents displayed a strong preference for dilute sea water over fresh water. A possible explanation for this apparently anomalous behavior may lie in a preference reaction directed toward a medium in which the fish are required to do the least amount of osmotic work. It will be remembered that the concentration of the dilute sea water tested in this series of experiments was close to the isosmotic level for fresh water fish (Table I). While a similar explanation may hold for the response of chum and pink to *dilute* sea water, it cannot account for their preference for *concentrated* sea water.

The responses of pink fry were neither as marked nor as rapidly expressed as those of chum fry. This may not represent a real difference in salinity preference between the two species. Comparison of the activities of chum and pink fry showed that that of the pinks remained at a high level throughout the period of observation while that of the chums decreased during the final 150 minutes of observation. Sustained high levels of activity would reduce the apparent level of any response requiring for its expression concentration in a relatively small area. Differences in activity levels, and rates of accommoda-

tion to environmental stimuli, are factors which must be considered when comparing the responses of the three species to an environmental variable under the artificial conditions of an experiment of this type.

Although marked changes are known to occur in the physiology of anadromous fishes prior to migration there are few data which can be correlated with the behavior observed in this investigation. However, it has been frequently observed that juvenile salmon will die if held in fresh water for long after their normal migratory period. This is believed to be due to osmotic stresses caused by a change in the ability of the fish to regulate their water and electrolyte balance with respect to the tonicity of their medium. Stresses resulting from a gradual shift from fresh water to marine osmoregulatory processes, possibly due to seasonal changes in thyroid activity (1, 11), while the fish are in fresh water are thought to stimulate general activity (appetitive behavior) which through downstream movement of the fish leads to the sea where the stresses are alleviated (8). From this viewpoint initial contact with sea water is the releaser for the consummatory act—movement into sea water.

It was observed in most experiments that entry into sea water resulted in a reduction in general activity. Similar observations have been made by several authors; for example Huntsman and Hoar (12) found that Atlantic salmon smolts (*Salmo salar*) became quiescent for several hours after abrupt entry into sea water, and Shepard (16) demonstrated a decrease in the activity of chum salmon fry with increase in the salinity of their surroundings. More recent observations by the writer have confirmed this for chum fry. Whether this effect is due to some specific action on the neuromotor system or to a general change in behavior is unknown. However, since fry which had been acclimated to sea water for 24 hours before testing did not show as great a decrease in activity, the effect may be in some way associated with final adjustment to the more concentrated medium. Lowering of activity on first entry into sea water may enhance the movement of the fish seaward through passive displacement by outgoing currents.

A further point of interest concerns the preference displayed by chum and pink fry and by coho smolts for the compartments in which they were first placed during control experiments. This behavior was most marked in chum fry, less apparent in pink fry and coho smolts, and apparently absent in coho fry.

These species may have the ability to apprehend and remember spatial relationships. Preference for the original compartments might then be the result of preference for "recognized" areas. Recent experiments by Hoar (10) suggest that chum and pink fry can rapidly learn direction in circular channels and retain this knowledge for some time. In the present experiments all compartments were of uniform size and color but visual cues may have been present in the position and form of aerators, supply lines, and sampling tubes. In coho fry, which exhibit a high degree of territorialism, recognition of area must be an important feature of behavior. That this species alone did not

exhibit the preference might seem to obviate this hypothesis but it must be remembered that their territorial drive would tend to produce scattered distributions.

An alternative suggestion to account for this behavior entails the formation of schooling odors by the fish. Göz (5) and Keenleyside (13) have demonstrated such odors in various species and shown their action to be significant in the initiation and maintenance of schooling. As yet no similar investigation has been carried out on Pacific salmon, but their marked tendency to school may possibly be due in part to the formation and release of such substances. If this is the case the attraction of the compartments in which the fish were first placed would lie in the relatively high concentration of schooling odors in the water. Such a theory accounts for the presence of this preference reaction in chum and pink fry and coho smolts, and its absence in coho fry. But whatever the nature or natural significance of this reaction it is apparent that it had little influence over the salinity responses of the fish.

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THE ROLE OF CLIMATE AND DISPERSAL IN THE INITIATION OF OUTBREAKS OF THE SPRUCE BUDWORM IN NEW BRUNSWICK

II. THE ROLE OF DISPERSAL[1]

D. O. GREENBANK[2]

Abstract

In Part I of this paper consideration was given to the role of climate in the initiation of outbreaks of the spruce budworm in New Brunswick. Analysis of the available weather data showed that the 1912 and 1949 outbreaks developed after several consecutive dry summers. Support was given to the theory of climatic release, which explains the time and place of outbreaks on a climatic basis. However, the recorded history of the spruce budworm also shows that high populations appeared in New Brunswick shortly after "spreading" through Quebec, and this suggests that the New Brunswick outbreaks are also a continuation of this spread. In the present part of the paper consideration is given to the role of dispersal. Moth dispersal is a more effective agent of spread than larval dispersal. Moths may be transported by convectional and turbulent air currents for long distances. Light traps used to detect the incidence of moth movements, showed that large segments of a population may be transferred from one area to another. Unspent females often predominate in these movements. Moth invasion was not detected before the 1949 outbreak although there is evidence from other sources that it occurred in 1948. When deposited in dense, mature, softwood stands, the moths can create outbreaks, but when deposited in young, open, or mixed-wood stands the ensuing high populations soon decline unless bolstered by repeated invasions. Populations in New Brunswick showed gradual and general increases as early as 1947. It is thought probable that these increases resulted from the build-up of local populations through climatic release. The nearest highly-populated centers were over 100 miles to the west in 1947. Later, invasion of moths from centers outside of the Province may have hastened the process.

Introduction

It is perhaps natural that the Green River Project, created to further our understanding of the epidemiology of the spruce budworm, *Choristoneura fumiferana* (Clem.), should encounter the problem of whether an outbreak develops independently within a region from increases in the local population or whether it results from the spread of an already existing infestation. This is the second of a two-part paper discussing the role of climate and dispersal in the initiation of outbreaks of the spruce budworm in New Brunswick. Whereas Part I discussed climate and the "discreteness" of outbreaks, Part II deals with the dispersal of populations.

Forest Insect Survey collections show that the spruce budworm, rather than disappearing altogether from New Brunswick, was present in small numbers after the 1912–1920 outbreak. Significant increases in this endemic population were first detected in 1947 but there is no assurance that increases

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did not begin earlier (11). By 1949, populations had risen sufficiently to cause noticeable defoliation, and 1949 is referred to as the "outbreak year" of the present infestation just as 1912 was the "outbreak year" of the former infestation. In the previous paper (5), analysis of weather data showed that the 1912 outbreak, which was widespread throughout the province, began after several summers of generally dry weather. The 1949 outbreak, which was confined to northern New Brunswick, also began after that region had experienced several successive dry summers. These findings support the theory of climatic release, which explains the time and place of outbreaks on a climatic basis (17).

Climatic release appears to act generally throughout the region experiencing the favorable weather, and the initial increases in population occur in both mature and immature stands. Subsequent increases, however, take place at a greater rate in the mature stands. Outbreaks gain their momentum in extensive areas of mature balsam fir and it is in such areas that the first severe defoliation is generally noticed. This pattern of development of outbreaks is not always consistent, however, and stands of the so-called non-susceptible type may be severely defoliated early in the outbreak history. Dispersal, which can concentrate populations in areas of deposition, is shown here to be the causal agent of such discrepancies.

Once dispersal is accepted as a factor operating in the course of an outbreak, it immediately becomes suspect that the outbreak arose through the spread of populations into the affected area. The past two outbreaks appeared in New Brunswick a few years after others developed in areas to the west. New Brunswick, situated in the major exit channel of North American weather systems, is at the receiving end of dispersal from these infested areas. Thus population increases in New Brunswick during the preoutbreak years might have resulted from, or at least been augmented by, invasion. Forest Insect Survey maps show that the current infestations in Quebec occurred farther and farther eastwards in successive years and gradually approached the borders of Maine and New Brunswick (4). Approximate distances of these areas of heavy infestation from New Brunswick were:

1945	250 miles
1946	200 miles
1947	175 miles
1948	50 miles
1949	Outbreak year in northern New Brunswick

The purpose of this study is therefore to decide whether outbreaks of the spruce budworm would have developed in the susceptible forests of New Brunswick in response to the relaxation of climatic control regardless of whether centers of heavy populations had existed in areas to the west. Furthermore, by describing the different types of dispersal and their magnitude, this paper prepares the way for a more detailed contribution from the Green River Project which will describe dispersal as an important mortality factor in the population dynamics of the budworm in different stand types.

Larval Versus Moth Dispersal

The spruce budworm is a vagile insect, the larva being eruciform and the adult winged. Voluntary dispersal, which is limited to short distances because of the length of time active crawling or flight can be maintained, is directed towards the everyday needs of finding food, avoiding enemies, and living where the preferred conditions exist. The shift of larval populations from one crown level to another with the depletion of food is evidence of voluntary dispersal. Also, the uneven distribution of egg populations within dense stands which results from the tendency of moths to congregate around the taller and more exposed trees on which they prefer to oviposit is another example (2, 10). However, by its behavior the budworm exposes itself to involuntary dispersal by air currents and may be transported over distances of several miles.

First-instar larvae after hatching from the eggs usually wander on the foliage for a brief period before settling in overwintering sites. Similarly, in the spring, second-instar larvae on emergence from hibernacula wander before establishment in feeding sites. During these periods, the tiny larvae often drop on silk threads and are carried away by the wind. Such dispersal, although initiated by the behavior of the insect, is involuntary and passive; involuntary because the dropping on silk threads is commonly caused by mechanical disturbances, such as the whipping of branches, and passive because the larvae, once the transport has begun, have no control over the direction or duration of movement. Adults are also subject to long-range dispersal. Moths in normal flight activity above the forest canopy may be swept away by air currents.

Three phases must be successfully completed if dispersal is to play a major role in the initiation of outbreaks. Individuals must first of all be transferred from one place to another; secondly the individuals must establish themselves in the new locality; and finally the species must establish itself. In these respects dispersal in the adult stage is more effective than dispersal in the larval stage. Each moth is active in the field over a period of about 12 days and is therefore more subject to dispersal than first- and second-instar larvae which are exposed for only a few hours. Because of their ability to fly in search of their host, adults have more chance of successful establishment where they are deposited. Larvae fall where they may, whether it be over lakes, host, or non-host vegetation, and their wastage is far greater. Also, larvae, even when deposited over host vegetation, have to reach maturity before dispersal becomes effective. Mated females, on the other hand, carry with them immediate powers for the perpetuation of the race in the new environment. Relative efficiencies of larval and moth dispersal are evident in the life tables of the spruce budworm. First- and second-instar larval dispersal has always given rise to a high mortality and, for a given density of stand, this mortality has been remarkably constant from year to year (11). Egg populations in the same tables have often been significantly greater or less than expected, indicating deposition of moths into the area or drainage

of moths from it. Consequently, in this study of the role of dispersal in the initiation of outbreaks of the spruce budworm, consideration need be given only to moth dispersal.

Means of Moth Dispersal

The flight habits of the spruce budworm have been described by Henson (6). Moths become active in the evening and are seen flying around the tree tops in greatest numbers when the light intensity is decreasing rapidly. While in normal flight, the moths may be transported passively by conventional or turbulent winds for long distances. Females are not as active as males and do not fly until they have deposited some of their eggs. More recent work by Blais (3) suggests that undersize females from highly-populated areas are able to fly soon after emergence.

Long-range dispersal is essentially involuntary but under circumstances of population pressure it may have a voluntary aspect. Evidence from New Brunswick studies shows that females may not be as sluggish as had been believed previously and under certain weather conditions may be even more subject to dispersal than males. Also, in heavily-populated centers, where foliage is depleted, females may become more active in their search for oviposition sites, there being a definite tendency for females to oviposit on needles of the more recent years (10). With normal flight activity increased, the chances of long-range dispersal are increased also and a portion of the population may be removed from the area.

Convectional Transport

Phenomenal numbers of spruce budworm moths have frequently invaded towns that were sometimes far distant from the nearest infestations (Fig. 1) Henson (7) examined these records in conjunction with weather maps and found that in practically all cases the invasion was followed by the passage of a cold front. He concluded that the strong updrafts associated with the prefrontal storm cells carried aloft and transported those moths that were active over the forest. Subsequent deposition of the moths in the central downdrafts would account for the sudden appearance of moths at centers over which the storm cells later passed.

During studies of the flight activity of the spruce budworm, actual depositions of moths from convectional storms have been observed at Green River. For example, on July 15, 1952, skies became overcast with threatening cumulus clouds in the early evening but comparatively few moths were seen flying until 2200 hours. Simultaneous with the second heavy downpour, the air became filled with moths. Spruce budworm and forest tent caterpillars (*Malacosoma disstria* Hbn.) were predominant amongst the thousands of insects that swarmed around the lights at the Laboratory. The heavy rain and moth activity continued until midnight when the sky overhead cleared and a bank of towering cumulus could be seen moving away to the southeast. This abnormal moth activity was not observed at lights a few miles distant.

FIG. 1. Campbellton, N.B., invaded by spruce budworm moths on night of August 6. 1956. (Photograph, courtesy Forest Protection Ltd.).

Furthermore, populations of the forest tent caterpillar were low in this area in 1952, suggesting long-range transport rather than an increase in local moth activity resulting from the unusual weather conditions. Weather maps showed that a cold front approached Green River from the northwest on July 15 and was moving at about 10 miles per hour in the late evening. This front was so orientated that prefrontal storm cells which passed over Quebec infestations of the spruce budworm and the forest tent caterpillar later passed over the Green River. Moth invasion was reported at several other points in New Brunswick during the night. These invasions were made up almost entirely of the spruce budworm and in no instance was the forest tent caterpillar recorded. It would seem that the storm cells, through their updrafts and downdrafts, repeatedly draw up and discharge insects as they pass over forested areas. Pressure and temperature fluctuations before and during the passage of storm cells could stir the moths into increased activity even after light intensities had decreased to a minimum. The distance that moths may be transported by convectional storms is no doubt variable. In 1952 the nearest forest tent caterpillar infestations were 25 miles to the northwest of Green River. On numerous other occasions large-scale moth invasion has been observed in New Brunswick.

Convective currents sometimes become vigorous in the afternoon under clear air conditions. Depending upon the altitude to which this activity continues, local air-mass thunderstorms or cumulus cloud may develop. Such convection may act also as an agent of dispersal by carrying those insects already in normal flight up into the cloud, or at least by keeping them in suspension in turbulent eddies at a height well over the forest. With the diurnal decrease of convective activity these moths are deposited. Moth depositions have frequently been observed when these weather conditions have prevailed. However, dispersal through air-mass convection will usually be a shorter-range process than dispersal through frontal convection because the life of air-mass cumulus or cumulo-nimbus is relatively brief. This type of convection probably stirs up and mingles contiguous populations rather than introducing distant populations.

Dispersal through convectional transport, although moving populations in the direction of the weather systems or of the travel of individual cells, cannot, by its very nature, spread populations evenly over an area. Moths once absorbed by the convective currents may be transported several miles before being deposited. Air-mass thunderstorms tend to follow topographic features, and stands along their routes should have moths deposited in them more frequently than stands off the routes. To a lesser extent this also applies to the intensity of frontal cells. These become weaker or stronger according to topography so that certain areas of New Brunswick are more susceptible to invasion by convectional transport than others. In the early years of an outbreak an area of deposition may become a center of relatively high population and this provides another reason for the early appearance of severe defoliation in one stand and not in another.

In Europe, moth invasion was at one time commonly postulated as the explanation of outbreaks of the nun moth. The sudden appearance of feeding damage in restricted parts of a more or less continuously forested area led many observers to believe that moths had been borne aloft from distant centers of infestation, transported over many miles, and ultimately deposited. On deposition, the invading moths created new outbreaks that were separated from the original seat of the calamity. Today, forest entomologists in Germany argue against the theory of "moth invasion" and postulate that outbreaks develop from increases in the resident population. Localized differences in the microclimate and the biocenosis are now believed to account for more rapid increases in population in one place than another and for earlier damage in one part of the forest than another (12, 16). The theory of "moth invasion" is opposed chiefly on the grounds that, predominantly, males take part in the migrations and that the females participating have already deposited their eggs. These objections do not hold true for the spruce budworm as will be shown below.

Turbulent Wind Transport

Turbulent wind transport differs from convectional transport in that the moths, rather than being borne aloft by updrafts, are swept along in the sur-

face winds. It has been observed most frequently in the evening when moths in normal flight activity are sometimes caught in the stronger air currents within the frictional zone of the trees. On one occasion at Green River, winds of 20 miles an hour blew from the direction of heavily-populated areas and swept moths along a front extending as far as could be seen from a high tower. The phenomenon lasted for more than three hours and could be likened only to the spectacular migrations of locusts. Distances that moths are transported depend upon wind velocity and the topography over which they are being swept. It is, however, more of a local phenomenon than convectional transport and causes the gradual and continual spreading of populations in the downwind direction.

Local topography may make some areas more favored for deposition and others more favored for drainage of moths (11). However, it seems unlikely that wind dispersal could concentrate the moths of an endemic population in one spot in sufficient numbers to release a population from natural controlling influences. After climatic release has encouraged a gradual but general increase in population over a wide area, however, any tendency for the concentration of population in localized areas could be a very important factor in the initiation of the spot infestations which are the first apparent outbreak symptoms (5).

The Use of Light Traps

Most direct observations of moth invasion have been made at towns and cities. As these centers are not equally distributed throughout the province, northern and central New Brunswick being heavily forested and relatively uninhabited, a network of light traps was established in 1945 for the detection of moth invasions. These traps were set up at various vantage points, particularly at forestry lookout towers. In order to improve the coverage in isolated sections of the province, the number has been increased from five traps in 1945 to 26 traps in 1955. A Coleman gasoline lantern, the source of light in these traps, is suspended between two baffle plates. Moths that are attracted to the light hit the baffle plates and fall into a container where they are killed by potassium cyanide fumes. At each location a trap is set up a few feet above the ground and the lantern lit every evening throughout the adult period. The catch is recorded the following morning when the light is extinguished.

A catch is a sample of the night-flying photopositive moths that are active in the vicinity of the light trap. The size of catch depends upon the number available for trapping. On nights when the locality of a light trap is invaded the number available is increased and catches larger than normal are made. Large-scale invasions can thus be detected from an examination of the size of catches. However, no matter how many budworms are transferred from one area to another, the transfer cannot affect the trend of the resident budworm populations if only males take part or if the participating females

are spent. From a more detailed examination of light-trap catches, numbers transferred can be translated into population transferred and the true importance of invasion assessed.

Detection of Invasion

Size of catch is a particularly useful indicator of invasion when invasion takes place through convectional transport and the participating moths are deposited in a body over a small area. There are several additional aids for the detection of invasion by convectional transport. It begins only under certain weather conditions and should be suspected when cloud types in the late afternoon indicate convective activity and especially when this activity is accompanied by thunderstorms. The inclusion in the nightly catch of a species foreign to the area is of course an indicator. Also, moths taking part in large-scale invasions usually originate from areas of infestation where one species predominates and the proportion of this species in the total catch of Lepidoptera will be very high. The catch of July 28 made by a trap at Green River in 1949 was taken as representing an invading population not only because of the large number of budworm moths trapped but also because of the predominance of this species in the total catch of Lepidoptera.

Night	Total catch of Lepidoptera	No. budworm moths	% budworm moths
July 26	245	0	0
" 27	340	10	3
" 28	2,293	1,520	66
" 29	247	25	10
" 30	150	20	13
" 31	73	0	0
Aug. 1	29	1	3

Simpson (14) has recently summarized the statistics of the New Brunswick light-trap catches. The number of spruce budworms taken each year by the light traps and the number of collections are recorded in Table I. A collection is taken to be a catch containing one of more specimens of the budworm, made by a trap on any night. Also, budworm collections have been broken down by size. In the first two years of operation the budworm was not taken. Traps in the northwest in 1947 and several traps in northern and central New Brunswick in 1948 yielded collections. These, however, were all small, less than 10 on any night, and no doubt came from local rather than invading populations. Nevertheless, moth invasion could have occurred in these preoutbreak years and gone undetected, for few light traps were in operation. For example, it is thought probable that the lower Quisibis region was invaded in 1948. The stands there are relatively immature and contain high percentages of hardwood. Such stands are not considered favorable for the rapid increase of resident populations. Nevertheless, this along with other areas of northern New Brunswick showed severe defoliation in 1949, the outbreak year of the present infestation. The high populations in areas

of mature balsam persisted and gained momentum in the years following but the Quisibis populations declined.

Moth invasion was detected for the first time from catc hesin light traps in the summer of 1949. In one catch at the Green River Laboratory, 1520 budworm moths were taken as compared with a previous high of 28 for that year. A cold front had approached from the northwest, the direction of budworm infestations 25 miles away in Quebec, and heavy thundershowers during the night indicated strong convective activity. In the summer following the invasion, two stands in the northern part of the Watershed showed unexpectedly severe defoliation while, in other stands, populations that had been increasing gradually since 1947 rose more abruptly. Again, this suggests that the rate of development of the outbreak was hastened by moth dispersal from outside areas.

Local populations available for trapping increased after 1949 as the outbreak developed in the Province and collections on the average became larger. By 1951 a few collections over 100 were being made but none was high enough to suggest mass invasion. In the following years several large collections over 1000 and a few over 10,000 were made, especially in 1952 and 1955. Because most of these came from traps located within sprayed areas and traps in the south of the Province where resident populations were small, they indicate invasion. However, these invasions need not have originated outside the Province, since populations within the Province were high enough to supply the necessary moths.

TABLE I

SUMMARY OF NEW BRUNSWICK LIGHT-TRAP COLLECTIONS MADE BETWEEN 1945 AND 1955

Year	No. traps	No. coll.	Coll./ trap	No. budworms	Budworms/ coll.	% distribution of coll. by size			
						<10	10–100	101–1,000	>1,000
1945	5	0	0.0	0	0.0	0	0	0	0
1946	4	0	0.0	0	0.0	0	0	0	0
1947	7	7	1.0	12	1.7	100	0	0	0
1948	7	14	2.0	37	2.6	100	0	0	0
1949	9	63	7.0	2,225	35.3	70	29	0	1
1950	8	65	8.1	302	4.6	97	3	0	0
1951	9	61	6.8	1,735	28.5	82	6	12	0
1952	14	205	14.6	85,522	413.1	50	26	17	7
1953	25	342	13.7	46,166	135.0	48	36	14	2
1954	26	230	8.8	30,788	133.3	39	37	21	3
1955	26	277	10.7	51,144	186.0	26	49	20	5

The Sex Factor of Invading Populations

Inordinately large light-trap collections strongly suggest that New Brunswick was invaded by the spruce budworm in 1949, the outbreak year, and in subsequent years. It is known both from direct observation and light-trap catches that phenomenal numbers of budworms may take part in these invasions but their effect on local populations cannot be estimated from

magnitude alone. The number of females partaking and their fecundity are the important attributes to be considered when assessing the likelihood of outbreaks being initiated by invading populations.

The fecundity of invading females can be determined from specimens caught in light traps. The sex factor of an invading population is more difficult to determine, however, because male and female budworm moths react differently to light. Thus light traps may take a larger proportion of the photopositive males and suggest a sex factor that is lower than that of the invading population. This difference between the sex factor of the population and the sex factor of trapped specimens can be determined in some instances for certain local populations where the sex factor is known. It was reasoned that this discrepance, the chances of trapping males over females through a sex difference in behavior, could be applied as a correction to the relative numbers of males and females trapped after an invasion, and the true sex factor of the invading population could thereby be estimated. In line with this approach, in 1953 two light traps were suspended from the crowns of balsam fir trees in a heavily-infested, dense softwood stand. The stand was not invaded and the local population of males and females was known. The results, below, indicate that the chance of trapping males over females is not constant but varies under different weather conditions.

Population Available for Trapping

Individuals of a generation of the spruce budworm do not develop at the same rate, and the appearance of adults in an area occurs over a period of several days. As a result, the abundance of moths available for trapping is constantly changing throughout the season. In 1953 the trend in the adult population was followed in the area where the light traps were located. The first adults emerged on July 7 and the last on July 25. Weather did not change markedly once emergence had begun and the distribution of the adult population in time was almost normal (Fig. 2A). Skewed distributions are to be expected when conditions change abruptly during the period of adult emergence. In 1953 the adult period lasted 30 days. The distribution curve shows that the abundance of moths available for trapping was at a peak in the middle of the adult period and fewer moths were available at earlier and later dates.

Over the 30 day period the two traps caught 28,898 spruce budworm moths, 84.3% of which were males. The nightly catches, which varied from 0 to 4750, have been expressed as percentages of this total and plotted in Fig. 2B.

The Sex Factor of the Population and the Sex Factor of the Catch

The sex factor is the ratio of females to the total population. The sex factor of the population in the stand was 0.45. This was determined from samples of empty pupal cases collected after adult emergence was completed. The sex factor of an adult population, however, changes from day to day throughout the adult period owing to significant differences in the rate of development and the longevity of the sexes. Rearing studies have shown that

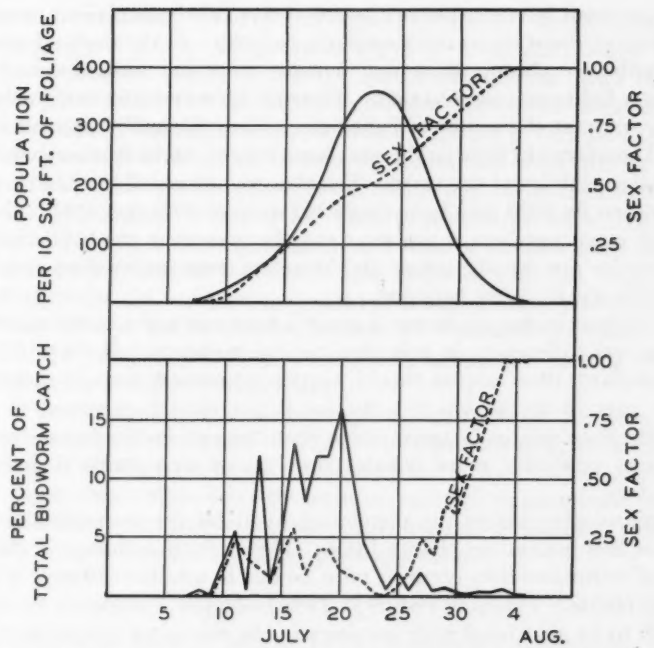

FIG. 2. A (above). The trend of the adult population in the field in 1953 and the sex factor of the population.
B (below). Nightly light-trap collections of spruce budworm moths expressed as percentages of the season's catch and the sex factor of these collections.

male larvae develop more rapidly than females, particularly in the sixth stadium (8). This sex difference in the rate of larval development is partially compensated for in the pupal stage which is briefer, by a few hours, for the female. The first adults appearing in an area are nevertheless invariably males. Females predominate at the end of the adult period because of the over-all slower rate of development in the immature stages and also because female adults live longer than males. Female longevity is, on the average, three days more than that of males. From these laboratory findings coupled with frequent field development checks in the pupal–adult period, the daily sex factor of the adult population could be determined. In Fig. 2A, both the sex factor and the moth population available for trapping can be read for any day during the adult period.

The sex factor of each nightly catch was determined (Fig. 2B). If males and females were equally active in the field and attracted equally to light, then the curve of sex factor of nightly catches should duplicate the curve of sex factor of populations available for trapping. It is, however, only at the beginning of the adult season, when only males are available for trapping, and at the end of the adult season, when, predominantly, females are available for trapping, that the two curves correspond.

Disagreement between the sex factor curves can be explained partially by the failure of the sexes to react similarly to light. In the dark-adapted state male moths are photopositive and females are either photonegative or else perform a light-compass reaction. Through light-compass orientation some females arrive at the source of light despite their normally negative response (17). Disagreement betwen the sex factor curves can be further explained by the relative activity of the sexes. Females are not usually as active as males and may not fly until they have deposited some of their eggs (18). Therefore, although equal numbers of the sexes may be present in the field, more males than females are usually active and therefore more males than females will actually be available for trapping.

Fluctuations in the sex factor of catches from one night to the next suggest that the sex differences in behavior are not constant. If these differences were constant, then catches should contain a preponderance of males for the greater part of the season but the sex factor should nevertheless increase gradually once relatively more males than females in the population began dying and relatively more females than males were being added to the population.

On consecutive nights the population available for trapping and the sex factor of this population change little. Therefore, if a change in size or sex factor of collections does occur, it must be due to weather differences between the two nights. Trapping results in 1953 indicated a tendency for increases in catch to be associated with increases in the sex factor of the catch. This tendency was again observed in 1954 when similar trapping experiments were conducted in another stand at Green River. The chi-square test showed the association of increase in size of collections with increase in the sex factor of collections to be highly significant. To explain this it must be postulated that certain weather conditions stimulate greater activity in budworm moths and that the degree of stimulation is greater for females than males. The sex factor of collections, even when females are active in greater numbers than males in the vicinity of the light traps is still limited by the photonegative behavior of females. It is unnecessary to postulate that the reaction to light by budworm adults changes under different weather conditions.

Just as the sex factor of collections may change from one night to the next, it can also change from one hour to the next in response to changes in weather. The sex factor is commonly highest in the early evening and becomes smaller as temperatures fall. Sudden weather changes have an immediate and pronounced effect on the relative activity of the sexes. In 1952, budworm moth activity was studied on two consecutive nights by focusing 300-watt spotlights on a large white sheet draped over a telephone line. Counts of each sex were made at periodic intervals (Table II). Fine weather prevailed on July 22 and the number of budworm moths settled on the sheet began to increase at 2130 hours, reached a peak shortly after midnight, and then decreased until all activity ceased at dawn. Weather conditions were markedly different on July 23. A squall line preceding a cold front approached

from the northwest and thunder was frequently heard. Moth activity was relatively greater than on the previous night up until 2230 hours, when a heavy thundershower occurred and the moths disappeared from the area. The sex factor of the budworm moths on the sheet just prior to the rain was 0.71 and because of the differential response to light, it is very probable that the actual proportion of active females to males was even greater. It is thought probable that the disappearance of moths was due to their leaving the area in the convectional storm rather than to a cessation of activity. Inordinately large collections with high proportions of females were made by two light traps 60 miles to the east. This demonstrates that convectional transport of moths did occur on that night although it cannot be stated that the collected moths had originated within the Green River area. Other centers of high population lay on the route of storm cells passing over the Green River area and the two light traps.

There is good supporting data to suggest that most of the very large collections made by New Brunswick light traps represent samples of invading populations. Females have usually predominated in these collections (Table III). Because these collections were made on nights with convective activity there is little doubt that the sex factors of the invading populations were even higher than those suggested by the trapped specimens.

TABLE II

SPRUCE BUDWORM MOTH ACTIVITY ON TWO CONSECUTIVE NIGHTS WITH MARKEDLY DIFFERENT WEATHER CONDITIONS

Date	Time, hr.	No. budworms	Sex factor	Weather conditions: Air temp., °F.	Cloud cover in tenths	Cloud type
July 22	2045	0	—	71	2	Cumulus
	2100	0	—	70		
	2130	10	0.10	69		
	2200	20	0.40	68	1	Alto-cumulus
	2230	250	0.39	67		
	2300	540	0.42	66		
	2330	590	0.28	65		
	2400	960	0.10	64	0	
	0100	1,700	0.10	62		
	0130	2,100	0.12	59		
	0230	875	0.08	58		
	0330	843	0.10	57	0	
	0430	170	0.08	55		
	0500	20	0.05	54	0	
	0530	0	—	54	0	
July 23	2100	4	0.50	75	6	Alto-cumulus, thunder
	2115	17	0.12	73		Calm
	2145	131	0.62	72	8	Cumulo-nimbus, thunder, lightning
	2215	1,090	0.71	72		
	2230	0	—	74	10	Cumulo-nimbus, heavy rain
	2300	0	—	67	10	Cumulo-nimbus, rain stopped
	2400	0	—	67	10	Winds 15–20 m.p.h.

TABLE III

THE SEX FACTOR OF NEW BRUNSWICK LIGHT-TRAP COLLECTIONS CONTAINING OVER ONE THOUSAND BUDWORM MOTHS

Sex factor	No. collections	Av. no. budworms per collection
0.1	0	—
0.2	0	—
0.3	1	1,100
0.4	2	1,500
0.5	3	2,800
0.6	7	6,100
0.7	12	4,000
0.8	8	1,800
0.9	9	2,400
1.0	2	2,100

Fecundity of Invading Females

One of the largest collections made by a single light trap contained 19,500 budworm moths and this was made by a trap operated within an area that had been sprayed and had a very low residual population. The sex factor of this collection was 0.57. Even though the collection was only a minute sample of the moths deposited on one night in the vicinity of the trap, the invaders would not contribute to the local population unless the participating females carried eggs.

Samples of females taken alive from invading populations have been set up individually in oviposition cages and the foliage later examined for fertile eggs. The results show that most of the transported females are partly gravid and that only 14% of those collected failed to lay any eggs. Field populations at Green River have an average fecundity of 200 under especially favorable circumstances but lower fecundities are more common especially where there is competition among larvae for food (11). Size of female moth as measured by the wing span has been found to be related to fecundity and this relationship is particularly useful in determining the portion of the reproductive potential that dispersing females are capable of carrying. The actual number of eggs laid by a female collected from an invading population may be expressed as a percentage of the original fecundity of that female as determined from the wing span/fecundity relationship. A few females collected from invading populations have laid over 50% of their original egg complement upon deposition. The mean number of eggs laid and the mean percentage of the original fecundity carried by four dispersing populations are listed in Table IV.

The Effect of Dispersal on Local Populations

Dispersal through convectional transport will generally have a greater effect on local populations than dispersal through turbulent wind transport.

TABLE IV

THE MEAN NUMBER OF EGGS LAID BY FEMALES TAKEN ALIVE FROM DISPERSING POPULATIONS AND THE PERCENTAGE OF ORIGINAL FECUNDITY CARRIED BY DISPERSING FEMALES ESTIMATED FROM FECUNDITY WING-SPAN RELATIONSHIP

Source	Mean no. fertile eggs laid	No. females tested	Mean wing span, mm.	Original fecundity	% fecundity carried by females
Convectional transport, Green River, 1952	40 ± 4	60	20.0	160	25.0
Convectional transport, Green River, 1955	47 ± 7	19	20.8	163	28.8
Convectional transport, Green River, 1956	41 ± 2	156	19.5	149	27.5
Turbulent wind transport, Green River, 1953	23 ± 2	57	18.5	139	16.5

With the former a portion of a local population may be transferred from one area and deposited bodily in another, whereas turbulent wind transport tends to spread local populations over wide areas. Also, more females and females with higher egg complements may be expected in convectional transport because prior to such transport females appear to be stimulated into greater activity.

One way of detecting moth dispersal and determining the effect on local populations is by means of an E/F ratio; the ratio per unit area of foliage of actual egg population to the number of female pupal cases showing successful adult emergence (9). E/F ratios can be computed for stands where population counts of pupae and eggs are obtained from the same or adjacent trees. In a non-starved population and under weather conditions ideal for larval feeding and development, if all females laid a full complement of eggs, an E/F ratio of approximately 200 would be expected. This is never realized in a resident field population since adult mortality would reduce this figure. Therefore if an E/F ratio of 200 or more is obtained for a plot and particularly if this is duplicated on other plots in the same area and in the same year, the conclusion that moth invasion has supplemented the resident population is well founded. In the Green River area inordinately high E/F ratios were recorded in several stands in 1949, 1952, 1953, and 1955 (Table V).

The effect of dispersal on local populations was clearly seen in New Brunswick when extensive areas of heavily-infested forest were sprayed from the air. In 1952, excellent spray coverage practically eliminated the budworm larvae over an area of 300 square miles. Although precautions were taken to minimize the threat of subsequent reinvasion by establishing spray boundaries wherever possible at natural buffer zones and at clear-cut or burned areas or mixed-wood stands, the spray area was more or less uniformly reinfested by moth invasion that same year (15). Wind dispersal from adjacent unsprayed areas and the convectional transport of moths from other centers of infestation brought egg populations up to 48% of those in comparable unsprayed plots (1).

TABLE V

THE RATIOS PER UNIT AREA OF FOLIAGE OF ACTUAL EGG POPULATION TO THE NUMBER OF EMERGED FEMALE PUPAL CASES ON THREE PLOTS AT GREEN RIVER 1949–1955

Year	Plot	Emerged female pupae	Eggs	E/F ratio
1949	G2 + G4	0.435	97.1	223
	G5	0.224	21.2	95
1950	G2	1.61	64.7	40
	G4	3.80	458.0	120
	G5	0.377	35.2	93
1951	G2	0.175	21.6	124
	G4	4.80	194.0	41
	G5	0.052	7.58	146
1952	G2	0.020	71.9	3,595
	G4	1.90	174.0	91
	G5	0.085	37.1	436
1953	G2	0.098	360.0	3,678
	G4	0.410	575.0	1,406
	G5	0.690	151.0	218
1954	G2	1.43	75.2	52
	G4	1.75	177.0	101
	G5	0.600	9.56	16
1955	G2	0.260	—	—
	G4	0.310	201.0	648
	G5	0.030	34.2	1,140

Discussion

The roles of climate and dispersal in the initiation of outbreaks of the spruce budworm in New Brunswick have been presented in the two parts of this paper. Part I was devoted to the analysis of the weather records for New Brunswick, which date back to 1887. Relatively dry, clear, early-summer weather, most favorable for budworm development, recurred in the years immediately preceding both the 1912 and 1949 outbreaks. Similar weather preceded other outbreaks in Ontario, Quebec, and Alberta. The theory of climatic release suggests that endemic populations of the spruce budworm throughout North America react to the relaxation of climatic control and that each regional outbreak develops independently of others.

Considerable data have recently been amassed on dispersal, and the second part of this paper has been devoted to the importance of dispersal in the initiation and course of an outbreak. The evidence that larval and adult populations are dispersed more or less continually, particularly in the direction of the prevailing wind, and that large segments of an adult population are transferred from one area to another is overwhelming. Because dispersing moths carry with them an adequate reproductive potential, moth dispersal must be accepted as an important factor in the epidemiology of the spruce

budworm. The immediate reinfestation of large areas in which resident populations had been practically eliminated by DDT is evidence of the ability of moth invasion to establish populations.

Gradual and general increases in population prior to the 1949 outbreak began at least as early as 1947. If invasions of insects from outside New Brunswick were responsible for these increases, then dispersal over distances of more than 100 miles must be assumed because the closest sources of high populations were in central Quebec at that time. Although such long-range transport may not be impossible, light traps, which have proved valuable in the detection of large-scale moth movements, failed to reveal invasion prior to 1949. Furthermore, these increases occurred rather generally over large areas and can therefore be explained more satisfactorily by changes in the local endemic populations than by spot deposition of moths. There is evidence that spruce budworm fecundity increases and its mortality decreases when summer weather is optimum for feeding and rapid larval development. If this stituation is sustained for several years in succession then the budworm may reach a population level that is many times higher than the general endemic level.

Although favorable climate and the prerequisite, a susceptible forest, may be sufficient to explain the change in population from endemic to epidemic proportions, moth invasion undoubtedly may hasten the process. The release of populations from controlling factors sometimes may occur only in those locations where deposited individuals are concentrated. For example, egg populations at Green River after the deposition of large numbers of moths in 1949 were considerably higher than could be expected from the local female populations, and in the following year two areas of heavy infestation appeared.

The tendency for outbreaks to appear in New Brunswick a few years after outbreaks have occurred through Quebec is clear in the recorded history of the spruce budworm. This tendency may one day be explained satisfactorily on a climatological basis when our understanding of the circulation patterns of air masses is more advanced. It is already known that shifts in the circulation pattern may occur in a manner that permits favorable polar air to predominate over one region of the Boreal Forest while tropical air predominates over adjacent regions. Consequently, outbreaks do not appear simultaneously throughout the range of balsam fir. There are also instances of outbreaks having developed in regions far distant from existing infestations. For example, the Lake Nipigon outbreak in Ontario in 1940, the minor outbreaks in Cape Breton and Newfoundland between 1920 and 1953, and outbreaks of the two-year cycle spruce budworm in the Canadian Rocky Mountains (13) were discrete and independent.

The following conclusions may now be drawn from these studies in New Brunswick. A forest in which there are extensive tracts of mature and overmature balsam is the prerequisite and climate is the immediate cause of outbreaks of the spruce budworm. The spruce budworm responds to favorable weather through increased fecundity and probably also increased survival in

both mature and immature stands but it is first in the mature stands that populations gain their momentum and escape the endemic control factors. Later, stands of all types may become severely infested through dispersing populations provided that the dispersal into these stands is sustained. Both favorable weather and moth deposition are ineffective in creating outbreaks if the forest is in a diversified condition. The effect of increased fecundity during the severe drought period 1922–1928 would not have been sufficient to overcome dispersal losses. Stands at that time had not recovered from the severe damage caused by the preceding outbreak, and the chances of successful establishment of dispersed individuals were consequently small. Moth invasions from heavily-populated areas to the west of New Brunswick may hasten the build-up of local populations but these are not essential to the initiation of outbreaks providing the favorable forest and climatic conditions exist. Moth dispersal plays an important role in the course of an infestation. Large segments of a female population, carrying an adequate reproductive potential, may be transferred from one area to another.

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THE TAXONOMIC STATUS OF RICTULARIA AFFINIS JÄGERSKIÖLD, 1909, RICTULARIA CAHIRENSIS JÄGERSKIÖLD, 1909, AND RICTULARIA SPLENDIDA HALL, 1913[1]

HAROLD C. GIBBS[2]

Abstract

The taxonomic status of *Rictularia affinis*, *Rictularia cahirensis*, and *Rictularia splendida* is discussed. On the basis of marked similarity between the characteristics used as differential criteria *R. cahirensis* and *R. splendida* have been made synonyms of *R. affinis*.

According to Dollfus and Desportes (1) about 30 species of the genus *Rictularia* have been described, but it is very uncertain how many of them are synonymous. In a number of species the male is unknown and the characters of the female, even when a full description is given, are frequently unsatisfactory for the separation of species.

Jägerskiöld (3), Hall (2), and Seurat (9) have suggested that the many species could be divided into two main groups, those parasitic in Carnivora and those parasitic in Rodentia, Cheiroptera, and Insectivora.

Hall (2) stated that in the species parasitic in carnivores the comblike cuticular structures of the anterior portion of the body of the female change very gradually into the spinelike structures of the posterior portion of the body, with noticeable alteration in the immediate vicinity of the vulva. In those species parasitic in rodents, insectivores, and bats, the comblike structures anterior to the vulva become spinelike posterior to it, the transition being marked in this area.

Jägerskiöld (3) in differentiating *R. affinis* and *R. cahirensis* stated that the vulva was anterior to the end of the esophagus in *R. affinis* and posterior to the posterior extremity of the esophagus in *R. cahirensis*. In his key, Hall (2) made the same distinction and added that in *R. splendida* the position of the vulva is similar to that in *R. cahirensis*. Sandground (7) considered this character subject to variation in specimens of *R. affinis* which he obtained from *Canis adustus*.

Hall (2) indicated that the length of the females of *R. affinis*, *R. cahirensis*, and *R. splendida* made an ascending series in which the maximum length of the smaller species was the minimum of the next larger. He gave the following measurements:

	Length of female, mm.	Egg size
R. splendida	8.37–10.55	38–42 × 32–34 μ
R. cahirensis	10.5–13.5	39–42 × 26–28 μ
R. affinis	13.5–20.5	36–38 × 24–26 μ

[1]Manuscript received March 14, 1957.
Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.
[2]C.I.L. Wildlife Fellow.

TABLE I

DISTRIBUTION OF SPINES ON FEMALES OF *R. affinis*, *R. cahirensis*, AND *R. splendida*

Species	Total no. of pairs of spines	No. of prevulvar pairs	No. of postvulvar pairs	Position of pairs of spines opposite vulva	Author
R. affinis	127–137	48–55	75–82	—	Jägerskiöld (3)
	140 and 151	51 or 52	—	—	Sandground (7)
	135	47–49	—	After 47–49	Seurat (8)
	146	57	58	58	Seurat (8)
	130–135	46–47	—	—	Kobulej (4)
R. cahirensis	126–135	46–52	78–84	—	Jägerskiöld (3)
	130	49	81	Between 49 and 50	Massino (5)
	136	49	87	Between 49 and 50	Massino (6)
R. splendida	136–138	—	—	55	Hall (2)

In comparing the number of spines recorded by various authors on different parts of the body of these three species, the present writer was impressed by the similarity in these values (Table I). This similarity and that between other characters used as criteria was so marked that it gave rise to doubt as to the validity of these three species.

In examining 49 specimens of *Rictularia* females from Egyptian foxes (*Vulpes* sp.) the writer found individuals which, by following the criteria given in Table I, he could place in either *R. affinis* or *R. cahirensis*. They are 12.4 to 30.1 mm. long. In some, the vulva is anterior, in others, posterior to the posterior extremity of the esophagus. Egg sizes are 39–46 μ by 30–35 μ. Total spine counts range from 124 to 151. Vulva pairs of spines are from the 45th to 56th pair.

Having identified the individuals tentatively as either *R. affinis* or *R. cahirensis* the writer examined more closely the main features used in differentiating the females of these species, in an effort to judge their validity. If they represented a normal distribution of variation it was felt that the assumption could be made that one was dealing with a single species. The criteria considered were: total length of the worm, total number of pairs of spines, the position of the pair of spines opposite the vulva, and the position (anterior or posterior) of and distance between the level of the vulva and the posterior extremity of the esophagus. The values obtained were then plotted in the form of histograms, which are discussed below.

Total length of female (Fig. 1).—This histogram is skewed positively. The only explanation for this seems to be that the sample studied was small. The histogram does not, however, deviate markedly from that for a normal distribution of variants and there seems to be no evidence of bimodality.

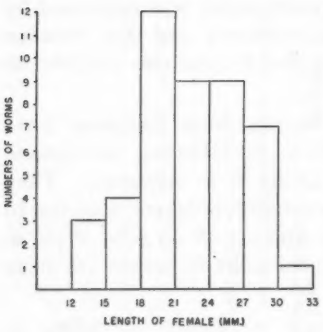
Arithmetic mean	=	22.3 mm.
Standard deviation	=	$\pm$4.2
Standard error of mean	=	$\pm$0.63 mm.

Total number of spines in female (Fig. 2).—This histogram is skewed negatively but the variations show a fairly close approximation to a normal distribution curve.

Arithmetic mean	=	135.69 spines
Standard deviation	=	6.48
Standard error of mean	=	0.98 spines

Vulvar pair of spines (Fig. 3).—This histogram shows the distribution of the position of the vulva in relation to the pair of spines opposite it, as indicated by the spine's number from the anterior end. This is an accurate figure and the histogram approximates closely a normal distribution curve.

Arithmetic mean	=	49.98 spines
Standard deviation	=	$\pm$2.32
Standard error of mean	=	$\pm$0.33 spines



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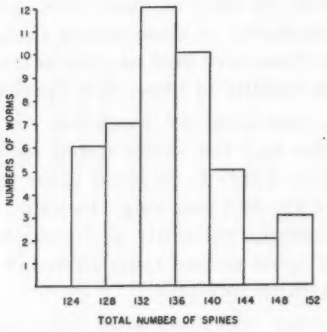

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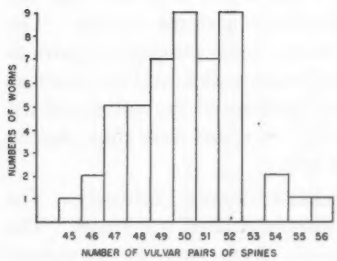

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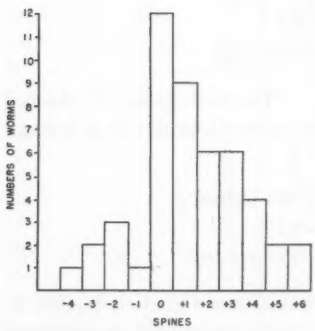

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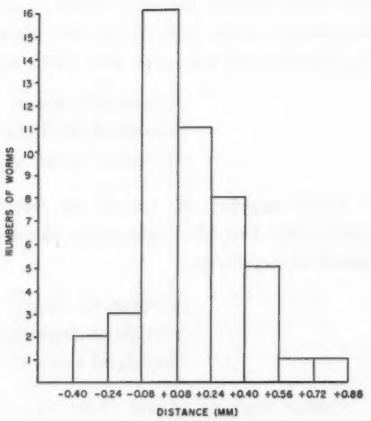

4

FIG. 1. Distribution of lengths of females. Class interval equals 3 mm. FIG. 2. Distribution of total numbers of spines per female. Class interval equals 4 mm. FIG. 3. Distribution of vulvar pairs of spines. FIG. 4. Distribution of distances from the posterior extremity of the esophagus to the vulva. Class interval equals 0.16 mm. FIG. 5. Distribution of number of spines from the posterior extremity of the esophagus to the vulva. Minus, vulva posterior: plus, vulva anterior.

Distances from the vulva to the posterior extremity of the esophagus (Fig. 4).—Initially this value appeared useful, as one of the main criteria for separation of the species was the position of the vulva in relation to the posterior extremity of the esophagus. In a few worms shrinking or wrinkling of the cuticle was noted. The esophagus is a fairly rigid structure and the vulva is attached to the cuticle. Therefore, in the event of much cuticular wrinkling the vulva tends to be displaced anteriorly to the end of the esophagus. This would partly explain the positive skewing of the histogram.

Arithmetic mean	=	0.15 mm.
Standard deviation	=	±0.23
Standard error of mean	=	±0.35 mm.

Number of spines between the vulva and the posterior extremity of the esophagus (Fig. 5).—As an alternative to measuring the difference in levels between the posterior end of the esophagus and the vulva, it was decided to make use of the differences in position of the spines opposite these levels. This histogram also showed a reasonably close approximation to a normal distribution curve but was positively skewed.

Arithmetic mean	=	1.2 spines
Standard deviation	=	±2.3
Standard error of mean	=	±0.34 spines

Although only 49 females were examined, the results convinced the writer that he was dealing with a single species.

Two few males were available for a critical analysis of their significant characteristics, although the following can be noted as a result of examining 20 of them.

Characteristic	Result
Total number of spines	97–113 pairs
Total length	Average 9.0 mm.
Spicule length	195–230 μ
Number of precloacal or mid-ventral fans	7–9

In Table II these results are compared with those of Jägerskiöld (3) for *R. affinis* and *R. cahirensis* and with those of Hall (2) for *R. splendida*. These figures show a marked similarity, the writer's being closest to those for *R. affinis*.

TABLE II

A COMPARISON OF THE MALE CHARACTERISTICS OF *R. affinis*, *R. cahirensis*, AND *R. splendida* WITH SPECIMENS FROM *Vulpes* SP.

Species	Total no. of pairs of combs	Total length (mm.)	Spicule length (μ)	No. of precloacal or midventral fans
R. affinis	111	7–8.5	220–230	6
R. cahirensis	96	4.8	170	7
R. splendida	108–109	4.83	207	8
Writer's specimens	97–113	9.0	195–230	7–9

Jägerskiöld (3) examined only one immature male of *R. cahirensis* so that his values for length and spicule length can be considered low. Hall (2) maintained the number of precloacal fans to be important in differentiating the three species: the present writer found the number of these to be inconstant in his specimens. Tiner (10) and Kobulej (4) considered this characteristic to be of little taxonomic significance. Hall (2) stated that one of the distinguishing characteristics of *R. splendida* was the possession by the male of three pairs of large conoidal preanal papillae. However, the writer considers the papillae described by Hall as conoidal, actually to be pedunculated papillae contained in a lateral ala. Seurat (8) suggested that *R. splendida* be placed in synonomy with *R. affinis*.

Table II shows that no one characteristic is sufficiently distinct to serve as a criterion for the differentiation of the species. Such differences as there are appear to be variations from a common mean. Initially, it appeared that in the female the position of the vulva in relation to the end of the esophagus would be a useful means of differentiation. However, among the writer's specimens both types of vulva were seen and the evidence in the histograms suggests that they are all members of a single species. Thus it appears that the main characteristic used in differentiating the three species is of doubtful value.

The three type specimens of these species were taken from three different hosts: *R. cahirensis* from *Felis* sp., *R. affinis* from *Vulpes* sp., and *R. splendida* from *Canis* sp. The first two are African parasites and the third is a North American parasite. Any existing slight morphological differences might, therefore, be attributed to environmental influences. The writer believes that these three species are conspecific. Accordingly, the specific name would be *Rictularia cahirensis* Jägerskiöld, 1909.

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PAPER CHROMATOGRAPHY IN INSECT TAXONOMY[1]

J. G. ROBERTSON[2]

Abstract

Seventeen species of Coleoptera, Lepidoptera, Diptera, and Hymenoptera showed chemical differences by the method of paper chromatography in an evaluation of the method for taxonomic purposes. The analyses were complicated by pattern differences evident in larval, pupal, and adult stages of some species. Geographic isolates of *Malacosoma disstria* Hbn. as well as of *Chamaepsila rosae* (F.) and physiological entities within *Pristiphora erichsonii* (Htg.) show identical patterns; however, differences are found at specific levels. Paper chromatography is therefore a valuable taxonomic tool, especially as the technique is capable of considerable refinement.

Introduction

Paper chromatography is an established microtechnique in a variety of scientific disciplines. A simple application of it to genetic problems was explored by Hadorn and Mitchell (4), who described chromatographic differences in developmental stages and mutants of *Drosophila melanogaster* Meig. squashed onto sheets of filter paper. Buzzati-Traverso (1) suggested that the method was suited to problems in taxonomy and population genetics after he found that it uncovered single gene and multifactorial differences in strains of *D. melanogaster*. Studies on fish (2) supported this suggestion. Kirk, Main, and Beyer (5) used filter paper disks to show chromatographic differences in seven species of land snails. In a study of the *Culex pipiens* complex, Micks (7) supplemented the technique by a chromatographic determination of amino acid extracts (8, 9). This method as used by Laven and Chen (6) revealed differences between normal and mutant strains of *C. pipiens*. Fox (3) recommended the squash technique (1) with two-dimensional paper chromatography as a result of his work on *D. melanogaster*.

This paper extends the above work by considering developmental, physiological, and geographic entities in 17 insects of the orders Coleoptera, Lepidoptera, Diptera, and Hymenoptera. No attempt was made to identify substances revealed by chromatography with specific chemical entities. However, as the ninhydrin reaction was used, proteins and their products, or both, are implicated.

Materials and Methods

Descending One-dimensional Chromatography

The species and their sources are shown in Table I.

Insects or their parts were squashed at 1-in. (2.5 cm.) intervals across Whatman No. 1 chromatographic paper 22½ in. (57.1 cm.) long and usually

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TABLE I

THE SPECIES STUDIED AND THEIR SOURCES

Species	Source
Coleoptera	
Cucujidae	
Laemophloeus pusilloides Steel and Howe	Ottawa, Ont.
Laemophloeus turcicus Grouv.	" "
Laemophloeus pusillus (Schönh.)	" "
Bruchidae	
Acanthoscelides obtectus (Say)	Belleville, Ont.
Lepidoptera: Heterocera	
Tortricidae	
Archips cerasivorana (Fitch)	Newton, Ont.*
Pyralididae	
Anagasta kühniella (Zell.)	Belleville, Ont.
Galleria mellonella (L.)	" "
Plodia interpunctella (Hbn.)	Ottawa, Ont.
Gelechiidae	
Gnorimoschema operculella (Zell.)	Belleville, Ont.
Lasiocampidae	
Malacosoma disstria Hbn.	Pitt Meadows, B.C.* Ste. Foy, Que.*
Malacosoma pluviale (Dyar)	Vancouver, B.C.*
Diptera	
Culicidae	
Aedes aegypti (L.)	Belleville, Ont.
Psilidae	
Chamaepsila rosae (F.)	Cambridgeshire, Eng.* P.E.I.* Holland Marsh, Ont.* Lulu Island, B.C.*
Sarcophagidae	
Kellymyia kellyi (Ald.)	Belleville, Ont.
Unidentified sarcophagid	From *M. americana* (F.), Belleville, Ont.*
Tachinidae	
Drino bohemica Mesn.	Belleville, Ont.
Hymenoptera	
Braconidae	
Macrocentrus ancylivorus Rohw.	Belleville, Ont.
Tenthredinidae	
Pristiphora erichsonii (Htg.)	Belleville, Ont.

*Field collection; others, laboratory cultures.

7 in. (17.8 cm.) wide. Several species were tested on each paper to overcome variation caused by chromatographic conditions such as temperature. As a chromatogram has a critical loading point, sufficient material to cover a circle of 0.50–0.75 cm. in diameter was used in each instance. To maintain this diameter, heads and halves of heads (cut medially) of larvae and pupae were often used. In *Laemophloeus* species four organisms were squashed at each starting point.

The paper was placed in a chromatographic tank (24 × 12 in.) or in a Chromatocab (Model 300A; Research Equipment Corporation, Oakland, California) adjusted for descending runs after the squash preparations had been dried for 3 hours or more. The total volume of a butanol – water – acetic acid mixture (4 : 5 : 1, V/V) was 400 ml. for tank development and 1400 ml. for Chromatocab development. The entire lower phase was used for equilibration and throughout the run. Volumes of 140 ml. (tank) and 840 ml. (Chromatocab) from the upper phase were used for irrigation regardless of the number of sheets being chromatographed. The paper was irrigated for 16–20 hours at 25–28° C. The solvent front was 46–49 cm. from the starting line under these conditions. The temperature for any particular run did not vary more than 1° C. *Chamaepsila rosae* (F.) was unavoidably run at 30–31° C.

The chromatograms were examined for fluorescent substances in a dark room with an ultraviolet mineral light that gave a dominant wavelength of 253 mμ. After the sustances and their color had been noted in pencil, the sheets were sprayed with alcoholic ninhydrin in aqueous collidine and developed with the aid of an infrared lamp.

R_f values were determined for the variety of substances revealed by the different methods and reagents. Buzzati-Traverso (1) considered the total number and positions of spots to be the basic pattern for judging differences at the genetic level. To overcome this interference, the basic pattern was considered to be the least number of replicable spots. For example, if an insect was replicated 10 times and substances could be detected with R_f values of .10, .20, .30, .40, and .50, with corresponding frequencies of 10, 10, 9, 2, and 1, the last two values were not considered in the ninhydrin analysis. They were occasionally included in the ultraviolet analysis because of a reduction in the number and frequencies of fluorescent substances. The R_f values among the various insects were assorted on the basis of size, locality, and relation to well-defined spots such as proline. Nevertheless, errors of assignment are probable, and substances with similar R_f values and color may or may not be identical. Accordingly, most differences are related to the number, color, and large differences in the R_f values of the substances.

Tables* were set up showing the standard error and the standard deviation of the mean R_f value of each substance. As these data were too extensive for reproduction here, they were reduced to formulae (Table II). Other differences could be noted, but discussion of these would serve no useful purpose at this time.

Ten amino acids, including *l*-proline and hydroxy-*l*-proline, were used to test the conditions of the experiments. As proline was the only substance that was detected in the insect chromatograms, it was used as a reference. In these experiments *l*-proline had a mean R_f value of .32 (n = 14, and S.D. ±. 015). The variation noted for proline is typical of the substances resolved from insect tissue.

*Copies of these tables may be obtained from the author.

Miscellaneous Tests

In descending chromatograms of *Laemophloeus* species, tests for reducing substances were made with ammoniacal silver nitrate (13).

Two-dimensional chromatography was also used with these species. Sixteen organisms (in eight pairs, back-to-back on the filter paper) were concentrated at the starting point. The first run was made in the butanol – acetic acid – water system, and the second in 80% phenol (V/V). The solvent front can be followed at all times when the solvents are used in this order.

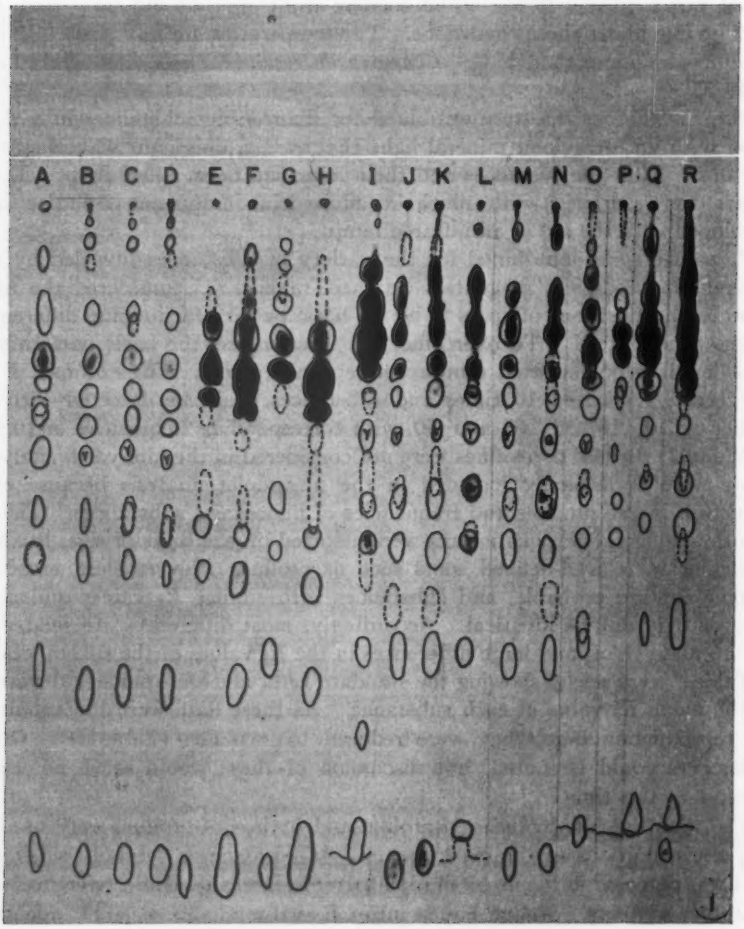

FIG. 1. Chromatographic patterns of insects. (The key to the capital letter heading each chromatogram is given in Table II. Spots within unbroken lines are ninhydrin-positive substances that include proline (Y) and substances whose colors are green (G) and brown (Br.). Spots within broken lines are fluorescent substances).

Results

Descending One-dimensional Chromatography

The chromatographic patterns for the various insects were made up of 8–11 ninhydrin-positive substances that usually fell into six groups (spots within unbroken lines, reading down, Fig. 1). Group I consisted of one to three spots that were small, had low concentration, and had R_f values of .04, .10, and .15; group II consisted of three to five spots that were large, had high concentration, and had R_f values of .17, .20, .24, .27, and .30; group III consisted of one yellow spot (marked Y in Fig. 1 and believed to be the amino acid proline because of its yellow color and its location) which had an R_f value of .32; group IV consisted of two spots that were diffuse, had low concentration, and had R_f values of .42 and .48; group V consisted of a diffuse spot which had an R_f value of .63; group VI consisted of one diffuse spot which had an R_f value of .89. In some cases the substances were difficult to localize because of streaking (Fig. 1, I and R). In Fig. 1 (F), an unmarked spot within the first broken line was evident. This was a result of pencil marking before ninhydrin development was complete for all substances.

The general ninhydrin pattern did not hold for the fluorescent analysis (spots within broken lines, Fig. 1), and fluorescent substances could not be placed into groups. It was difficult to be decisive in this analysis because of faint fluorescence of background material. However, particular concentrations of colors against the background were evident and formed a basis for many of the differences to be noted.

Table II shows that the tests distinguish between developmental stages. For example, differences were noted between early pupae, late pupae, and adults of *Macrocentrus ancylivorus* Rohw. and between the first- and third-instars of *Pristiphora erichsonii* (Htg.). Other tests show that it was presumptive to compare the head of one species with the whole or remaining portions of another species, and that differences exist between portions or organs (blood, gonads, Malpighian tubules, etc.) of a species.

Intergeneric differences were found in adults on the basis of the number of ninhydrin-positive substances and the presence or absence of proline. For example, the genera *Laemophloeus*, *Acanthoscelides*, and *Macrocentrus* had formulae with ninhydrin-positive portions of $11N_Y$, 10N, and 9N, respectively. The number of fluorescent components was the same for *Laemophloeus* and *Macrocentrus*, but differences were noted in the color and R_f values of particular substances.

Species at mid-pupal development showed 9–11 ninhydrin-positive substances. Proline was present in each case, except that *Drino bohemica* Mesn. had a substance of similar R_f value (.32) but green (G) in color. A brown substance was a feature of the head components of *D. bohemica* and of whole pupae of *C. rosae*. There was considerable interspecific variation in number, color, and corresponding R_f value of fluorescent substances among the pupae.

The chromatograms of larvae had 7–11 ninhydrin-positive substances. Only one species contained proline at this stage. The larvae were easily

distinguished to genera by the ninhydrin reaction. The variation in number, color, and corresponding R_f value of the fluorescent materials distinguished the species. In third-instar larvae of *P. erichsonii* a green substance (marked *c* in Fig. 1, R) was noted both before and after the ninhydrin reaction. Its R_f value was .94, and it is thought to be a plant pigment.

TABLE II

MAJOR DIFFERENCES AND SIMILARITIES IN CHROMATOGRAPHIC PATTERNS OF VARIOUS STAGES AND PORTIONS OF 17 SPECIES OF INSECTS

(N, ninhydrin-positive substances; F, fluorescent substances. Subletters refer to colors: blue (B), deep blue (DB), purple (Pu), brown (Br), blue-yellow (BY), green (G), yellow-green (YG), intense yellow green (YGG), yellow (Y), intense yellow (YY), and pink (P). Numbers preceding N and F refer to total number of substances; subnumbers give the mean R_f values for the corresponding subletters)

Species			Key letter (Fig. 1)
Adults			
Laemophloeus pusilloides	(whole)	$11N_Y$* – $5F_{BG.17}$	B
L. turcicus	(whole)	$11N_Y$ – $5F_{BG.15}$	C
L. pusillus	(whole)	$11N_Y$ – $5F_{BG.15}$	D
Macrocentrus ancylivorus	(male, whole)	9N – $5F_{BY.23}$	
M. ancylivorus	(female, whole)	9N – $5F_{BY.23}$	
Pupae			
Acanthoscelides obtectus	(whole)	10N – $3F_{DB.27}$	A
Archips cerasivorana	(head)	$9N_Y$ – $3F_{B.26}$	
Anagasta kühniella	(head)	$10N_Y$ – $5F_{BY.15}$	
Galleria mellonella	(head)	$10N_Y$ – $3F_{BY.23}$	
Gnorimoschema operculella	(whole)	$10N_Y$ – $8F_{Pu.47}$	
Malacosoma disstria, B.C.	(head)	$10N_Y$ – $4F_{BY.32}$	I
Aedes aegypti	(male, cephalothorax)	$10N_Y$ – $3F_{BG.57}$	J
A. aegypti	(female, cephalothorax)	$10N_Y$ – $3F_{Y.41}$	K
Chamaepsila rosae, England	(whole)	$10N_{Y,Br}$ – $4F_{P.04}$	L
C. rosae, P.E.I.	(whole)	$10N_{Y,Br}$ – $4F_{P.04}$	
C. rosae, Ont.	(whole)	$10N_{Y,Br}$ – $4F_{P.04}$	
C. rosae, B.C.	(whole)	$10N_{Y,Br}$ – $4F_{P.04}$	
Kellymyia kellyi	(head)	$10N_Y$ – $2F_{Pu.26}$	M
Unidentified sarcophagid	(head)	$11N_Y$ – $4F_{YGG.23}$	O
Drino bohemica	(head)	$10N_{G,Br}$ – $6F_{YY.41}$	N
M. ancylivorus	(early pupae, whole)	$11N_Y$ – $6F_{DB.26}$	
M. ancylivorus	(late pupae, whole)	$10N_Y$ – $7F_{Y.25}$	
Larvae			
A. cerasivorana	(head)	7N – $4F_{B.27}$	E
Plodia interpunctella	(head)	$11N_Y$ – $1F_{Y.36}$	
M. disstria, B.C.	(third instar, head)	9N – $4F_{Pu.12}$	F
M. disstria, Que.	(third instar, head)	9N – $4F_{Pu.12}$	G
M. pluviale, B.C.	(third instar, head)	9N – $5F_{YG.22}$	H
Pristiphora erichsonii, Man.	(first instar, whole)	8N – $3F_{Pu.16}$	P
P. erichsonii, B.C.	(first instar, whole)	8N – $3F_{Pu.16}$	
P. erichsonii, Man.	(third instar, whole)	12N – $6F_{Y.31}$	Q
P. erichsonii, B.C.	(third instar, whole)	12N – $6F_{Y.31}$	R

*Proline.

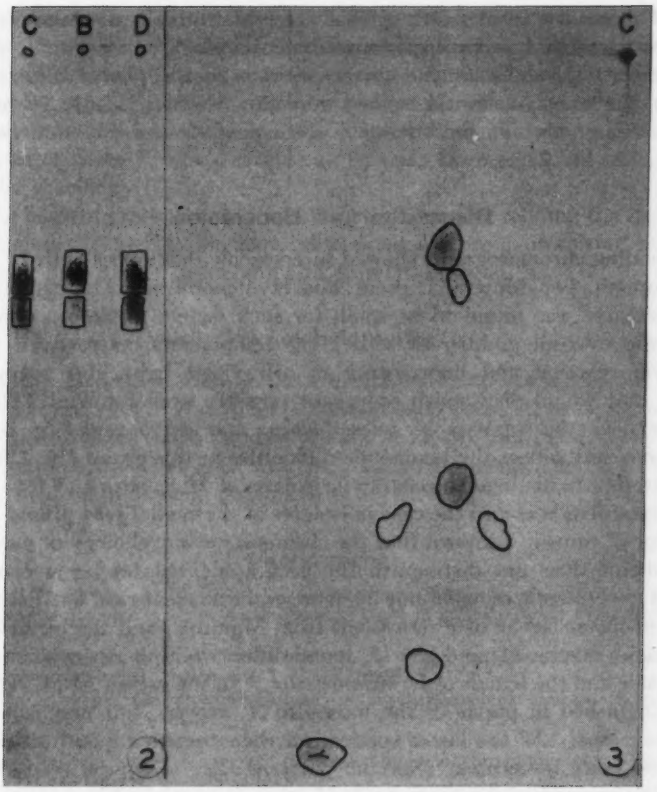

FIG. 2. Chromatogram showing reducing substances in *L. pusillus* (C), *L. pusilloides* (B), and *L. pusillus* (D) developed with ammoniacal silver nitrate.

FIG. 3. Two-dimensional chromatogram of *L. pusillus* developed with ninhydrin. Proline is indicated by the letter Y.

Miscellaneous Tests

It was noted in the previous section that the ninhydrin and fluorescent analyses by the one-dimensional method revealed interspecific distinctions except in *Laemophloeus* species. Fig. 2 shows that this difficulty could be partly overcome by use of the ammoniacal silver nitrate test. *Laemophloeus pusillus* (Schönh.), *L. turcicus* Grouv.,* and *L. pusilloides* Steel and Howe each had a reducing substance whose mean R_f value was .21. A second substance having an R_f value of .25 occurred three times in 12 trials of *L. pusilloides* but with 100% frequency in the other two species. Moreover, its concentration in *L. pusilloides* was visibly lower. As the two values corresponded with R_f values obtained by the ninhydrin reaction, it is possible that the two substances are amino sugars.

*Following the preparation of the manuscript it was learned that the material sent to Belleville as *Laemophloeus turcicus* Grouv. was mistaken for *Laemophloeus pusillus* (Schönh.)

Fig. 3 shows the results of a further attempt to distinguish these species. The substances that make up the two-dimensional chromatographic pattern gave similar R_f values for the three species. In the phenol direction, R_f values of the seven substances located were .26, .31, .55, .58, .61, .76, and .86. Corresponding values in the butanol – acetic acid – water direction were .22, .20, .19, .25, .14, .22, and .33.

Discussion and Conclusion

Descending chromatograms showed interspecific differences with 17 species of Coleoptera, Lepidoptera, Diptera, and Hymenoptera. The magnitude of the differences was found to be small for such widely separated categories. Hence the over-all number of separating components, as revealed by the ninhydrin reaction and fluorescence in ultraviolet light, did not provide criteria that would distinguish orders, or possibly even families. The need for extending the analysis of *Laemophloeus* species to tests for reducing substances may reflect the taxonomic difficulties in this group (10, 12).

An attempt to distinguish geographic isolates of *M. disstria* and *C. rosae* was not successful as was also the case in isolates of the snail *Theba pisana* (Müll.) (5). For *C. rosae* it is known that the chromosome morphology of metaphase complements does not distinguish the geographic isolates being considered here (11). Differences could not be detected in physiological entities of first- and third-instar larvae of *P. erichsonii* from Manitoba and British Columbia.

Buzzati-Traverso (1) and Fox (3) found different chromatographic patterns in the male and the female of *D. melanogaster*. In the present study, sex could be distinguished in pupae of the mosquito *A. aegypti*, but not in adults of *M. ancylivorus*. As the latter species is arrhenotokous a quantitative study might be more discerning. Specific patterns that might be related to sex were not apparent in chromatograms of other species in various stages.

In setting up comparative standards it is obvious that there is interference caused by differences in developmental stages and sex. A more serious difficulty is that imposed by the great disparity in size of insects as chromatography requires small amounts of material for resolution. In this study the head was commonly used because of its accessibility. In *D. melanogaster* abdomens were used by Buzzati-Traverso (1) and Fox (3) as Hadorn and Mitchell (4) found components of various eye pigments on their chromatograms. Interference due to eye color was not apparent in these studies.

Third-instar larvae of *P. erichsonii* provided the only evidence of a dietary component, and as this component could be recognized it did not interfere with the results. Diet did not interfere with the analyses of various *D. melanogaster* mutants (1), nor was it found to affect the musculature of *T. pisana* in experiments designed to test the importance of this factor (5). However, the possibility of this interference suggests that non-feeding pupae should be used in subsequent studies.

The disparity in size of the various insects imposes the greatest impediment to further work. This difficulty may be overcome by lyophilization or

homogenization of whole organisms. Such procedure would allow application of known amounts of material to the starting lines and separating components could subsequently be quantitated photometrically. Multiple analyses that involve solvent systems, reagents, and specific extractions should greatly refine the method and help in the identification of components. Prior hydrolysis of tissue is not desirable as this has been observed to cause loss in genetic specificity (1).

On the basis of present results and the possibilities in refining the technique, it is concluded that this biochemical approach offers a promising means of elucidating problems in taxonomy and population genetics.

Acknowledgments

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TAXONOMIC VALUE OF THE CONE TOP AND THE UNDERBRIDGE IN THE CYST-FORMING NEMATODES HETERODERA SCHACHTII, H. SCHACHTII VAR. TRIFOLII, AND H. AVENAE (NEMATODA: HETERODERIDAE)[1]

ROLAND H. MULVEY[2]

Abstract

Distinct morphological differences in the cone top and the underbridge of the cysts serve to separate *Heterodera schachtii*, *H. schachtii* var. *trifolii*, and *H. avenae*. Correlation between cyst volume and fenestral length of *H. schachtii* cysts was not significant. In *H. schachtii* the depth of the underbridge was significantly greater than in *H. schachtii* var. *trifolii*, but this difference may be of little taxonomic value because of the variation within the two forms.

Introduction

Three cyst-forming nematodes of economic importance in Canada are the sugar-beet nematode, *Heterodera schachtii* Schmidt, 1871, which attacks many cruciferous and chenopodiaceous crops (1); the clover cyst nematode, *H. schachtii* var. *trifolii* Goffart, 1932, which attacks many legumes (6); and the oat cyst nematode, *H. avenae* Filipjev, 1934, which attacks some graminaceous crops, especially oats (2). Recognition of these three cyst-forming nematodes is, therefore, very important, especially in mixed populations. Morphologically the cysts of these three forms are very similar. Franklin (5) summarized the literature on the morphological differences in shape, color, and the walls of cysts in *Heterodera*. Oostenbrink (7) used the length of the fenestra (transparent patches on either side of the vulval slit) and the vulval slit in separating *H. schachtii*, *H. schachtii* var. *trifolii*, and *H. avenae*. Cooper (3) used, in addition to the characters used by Oostenbrink, the presence or absence of an underbridge and the positions of the bullae (knoblike projections on the inside of the cone) in separating the three forms.

This investigation was undertaken to study lengths of the fenestra and the vulval slit of the three forms in Canada, the association between cyst volume and fenestral length, and the depth and structure of the underbridge.

Materials and Methods

The nematode material used was obtained as follows: of *H. schachtii* from greenhouse (Ottawa) cultures on the roots of cabbage, red beet, Brussels sprouts, rutabaga, sugar beet, and mangel; of *H. schachtii* var. *trifolii* from bean and pea grown in British Columbia; and from greenhouse (Ottawa) cultures on the roots of white Dutch clover; and of *H. avenae* from infested oat roots and soil in fields of southwestern Ontario.

[1]Manuscript received March 8, 1957.
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TABLE I

POSITIONS OF BULLAE, MEAN UNDERBRIDGE DEPTHS, AND AVERAGE MEASUREMENTS IN MICRONS OF THE FENESTRA AND THE VULVAL SLIT IN THREE CYST-FORMING NEMATODES

	Position of bullae	Underbridge depth	Fenestral length	Vulval slit length
H. schachtii	Well below fenestra	32.5	31.7 (64*)	44.5 (64*)
H. schachtii var. *trifolii*	Well below fenestra	30.5	45.3 (20)	47.4 (20)
H. avenae	Close to fenestra	No underbridge	43.2 (9)	12.9 (9)
Difference necessary for significance at 5% level		1.80		

*Number of measurements.

Permanent slide mounts of cones (the posterior part of the cyst) were prepared in the same manner as that described by Cooper (4). Cobb slides were very satisfactory in that the cone could be examined from the bottom as well as the top.

Cyst volume was calculated by multiplying the length of the cyst less the neck and the posterior protuberance by the square of the breadth. The depth of the underbridge was measured by focusing the microscope to the level of the fenestra, recording the reading on the scale, focusing down to the level of the underbridge and again recording the scale reading, and subtracting the two readings. Five measurements of each cyst were taken and the average was used in subsequent calculations.

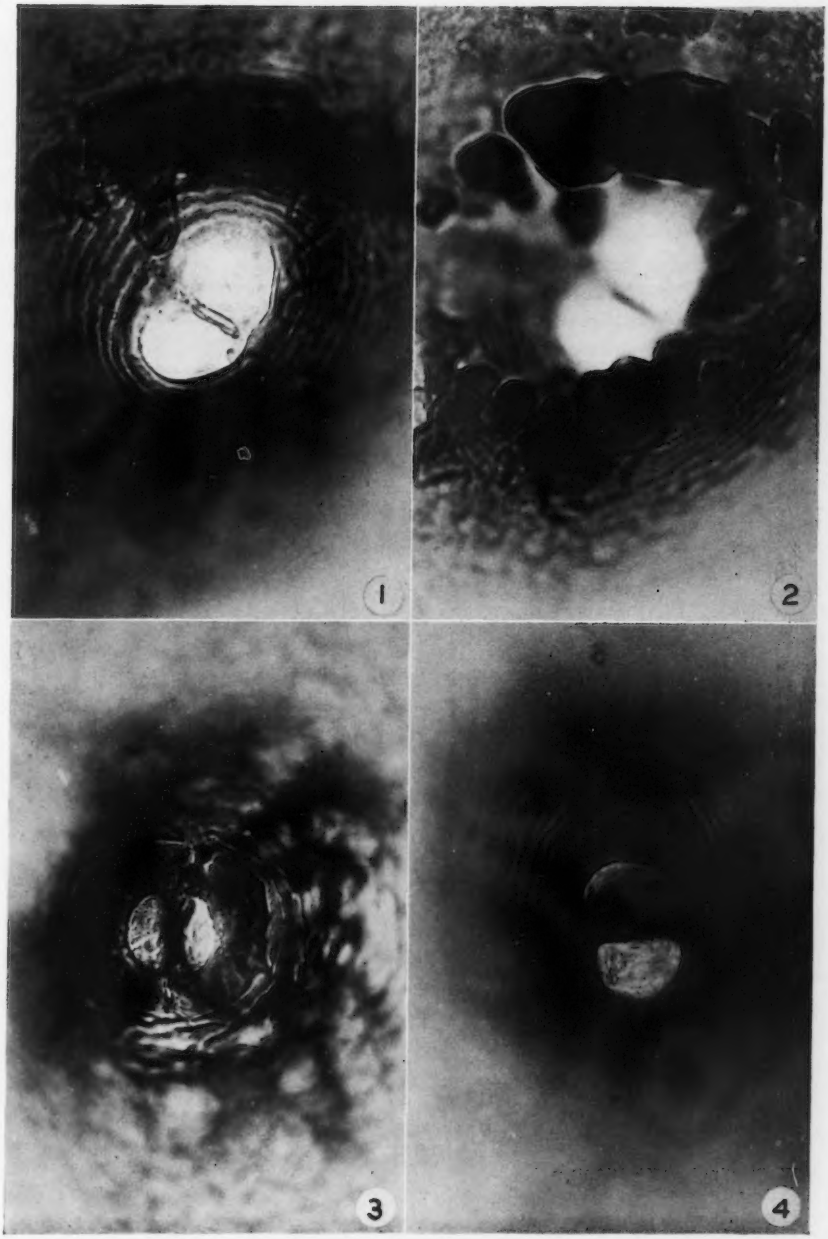
The lengths of the fenestra and the vulval slit were determined from camera lucida drawings of the cone tops.

Taxonomic Characters

A correlation coefficient of 0.290 for cyst volume and fenestral length of 36 *H. schachtii* cysts was not significant.

The average fenestral length in *H. schachtii* (31.7 μ, Table I) agrees well with Oostenbrink's (7) average of 32.1 μ for *H. schachtii* in Holland. Ninety per cent of the fenestral lengths of this form were within the range 24 to 36 μ. The fenestral length in *H. schachtii* var. *trifolii* ranged from 40 to 54 μ. Cooper (3) stated that he was unable to separate *H. schachtii* from *H. schachtii* var. *trifolii* by fenestral length because in his mounts of *H. schachtii* taken from beet roots the length was always more than 38.7 μ. The writer's findings coincide with those of Oostenbrink (7) in that *H. schachtii* shows smaller fenestra on the lip tops than *H. schachtii* var. *trifolii* and that this character may be useful for separating these forms.

FIGS. 1–4. Photomicrographs of cones of cysts. ×400.
FIG. 1. *Heterodera avenae*, cone top showing fenestra and short vulval slit. FIG. 2. *H. avenae*, bullae within the one. FIG. 3. *H. schachtii*, cone top showing fenestra and long vulval bridge. FIG. 4. *H. schachtii* var. *trifolii*, cone top showing fenestra and long vulval bridge.



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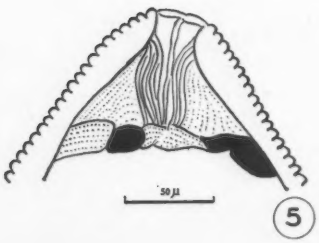

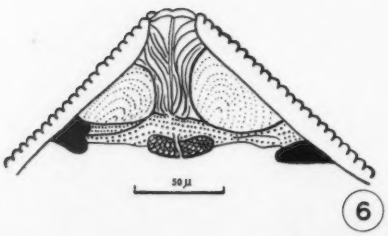

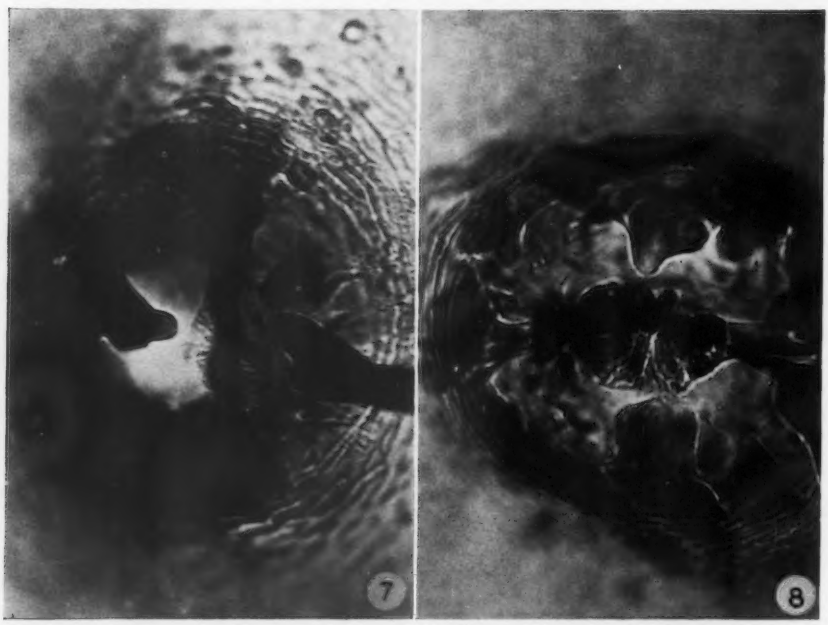

FIGS. 5, 6. Side view of cone showing underbridge, bullae, and sheaf-like vagina. FIG. 5. *H. schachtii*. FIG. 6. *H. schachtii* var. *trifolii*.

FIGS. 7, 8. Photomicrographs, anterior to posterior view of cone, showing underbridge, arms, and bullae. ×400. FIG. 7. *H. schachtii*. FIG. 8. *H. schachtii* var. *trifolii*.

In *H. avenae* the bullae (Figs. 1 and 2) were coarser and lay closer to the fenestra than in either *H. schachtii* (Figs. 3 and 7) or *H. schachtii* var. *trifolii* (Figs. 4 and 8). *H. avenae* cysts showed no underbridge (Fig. 1).

Thirty-six measurements of the underbridge depth of each of *H. schachtii* and *H. schachtii* var. *trifolii* were made to determine any differences. The underbridge depth of *H. schachtii* was greater at the 5% level than that of *H. schachtii* var. *trifolii* (Table I). However, since the measurements of the underbridge depth were only approximate, the value of this character in separating the two forms is doubtful. The underbridge in *H. schachtii* (Figs. 5 and 7) differs in size and shape from that of *H. schachtii* var. *trifolii* (Figs. 6 and 8). In *H. schachtii* it is more slender and less pigmented and the underbridge arms or ends do not bifurcate as they do in *H. schachtii* var. *trifolii*. These marked differences may be used in separating these two cyst-forming nematodes.

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BITING MIDGES (DIPTERA: CERATOPOGONIDAE) AS INTERMEDIATE HOSTS FOR HAEMOPROTEUS OF DUCKS[1]

A. M. Fallis and D. M. Wood

Abstract

Haemoproteus nettionis was transmitted to domestic ducks in late June and in July in 1954, 1955, and 1956. This coincided with the abundance of *Simulium rugglesi* and biting midges (Ceratopogonidae). Transmission of *H. nettionis* occurs during the night, at which time certain biting midges, which appear to be ornithophilic, feed on the ducks. It was shown experimentally that these midges (*Culicoides* sp.) are suitable intermediate hosts for *H. nettionis*. Oökinetes and structures identified as oöcysts and sporozoites were found in specimens of midges that were sectioned. *H. nettionis* was seen in the peripheral blood of ducks 14–21 days after they were infected. The gametocytes require 4 to 6 days to reach maturity.

Introduction

The mode of transmission and life history are known for few species of *Haemoproteus*. The Sergents (16) and Aragão (3) reported that the hippoboscid *Lynchia maura* Bigot was the intermediate host of *Haemoproteus columbae* Celli and Sanfelice, of pigeons; this was confirmed by Adie (1), who described the life cycle in the fly. The life cycle of this species was investigated also by Kartman (12), who reported oöcysts on the mid-gut of *Pseudolynchia canariensis* (Macq.). O'Roke (15), and more recently Tarshis (17), investigated the life cycle of *H. lophortyx* O'Roke, in the California valley quail (*Lophortyx californica* Shaw). They found also that hippoboscids were responsible for the transmission. Huff (11), using *Pseudolynchia maura*, a hipposcid of pigeons, transmitted *H. sacharovi* Novy and MacNeal to mourning doves, although he suspected, apparently, that *Pseudolynchia* was not the natural vector. Baker (4) reported, following studies of *H. columbae* in wood pigeons (*Columba palumbus* L.), that a species of *Ornithomyia* is a vector of *H. columbae* in England.

Haemoproteus has been observed in several species of ducks and geese by various investigators, including Herman (8), who brought the various reports together and concluded that the *Haemoproteus* of the Anatidae is *H. nettionis* (Johnson and Cleland, 1909). This parasite is known from the following ducks as a result of the examination of blood smears that were made during the past few years in different parts of Canada by various individuals: mallard duck, black duck, ring-necked duck, baldpate, wood duck, common goldeneye, surf scoter, pintail, green-winged teal, blue-winged teal, old squaw, and common merganser.* Many domestic ducks that were kept in recent summers in Algonquin Park became infected with *H. nettionis* as well as *Leucocytozoon simondi*. Since hippoboscids were reported to be vectors of other species of

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Contribution from the Department of Parasitology, Ontario Research Foundation, Toronto 5, Ontario, Canada.

*The common names used here are those given in Taverner, P.A. Birds of Canada, National Museum of Canada, 1934.

Haemoproteus it was assumed that a hippoboscid would be the intermediate host of *H. nettionis*. Hippoboscids were never found, however, on any of the several hundred ducks that were used in the study on *Leucocytozoon* (6), although in 1953 Anderson (2) examined these ducks daily to search for these flies. Moreover, Bequaert (5), after extensive studies of the Hippoboscidae, regards aquatic birds as unsuitable hosts for these insects. Therefore a more intensive search for the intermediate host of *H. nettionis* was undertaken. We began this investigation by noting when the parasite appeared in the blood of ducks that were placed outside at different times throughout the summer and relating the presence of the parasite to the prevalence of blood-sucking insects that were feeding on the ducks. The suitability of various groups of insects as intermediate hosts was tested by injecting ducks with suspensions of these insects and noting whether the ducks became infected. These data, showing that biting midges are suitable intermediate hosts for *H. nettionis*, are reported herein.

Materials and Methods

The work was done in Algonquin Park, Ontario, at the Wildlife Research Station of the Department of Lands and Forests, where *Haemoproteus*, as well as *Leucocytozoon*, was known to occur. White Pekin ducks, 2 to 3 weeks of age, were exposed to possible infection with *Haemoproteus*, by placing them out-of-doors from late May to September; blood smears were prepared from these exposed ducks at various times to determine the time at which they became infected with *Haemoproteus*.

Blood-sucking insects were collected from ducks by placing over the birds, at intervals throughout the day and night, a cage covered with nylon mesh (40–50 mesh per inch); black flies were collected during the day and biting midges and mosquitoes at night. The cage was left over the ducks for 20–30 minutes during which time insects on the ducks had time to complete their blood meals and fly to the sides of the cage. The insects were removed in an aspirator and kept in small cages (2). The method was modified slightly to capture biting midges and mosquitoes by keeping the ducks in boxes that were open at the top. This confined the ducks to a small area and they were only slightly disturbed when the cage was placed over them. A light was placed beside the cage when the midges and mosquitoes were being removed from it. Additional mosquitoes and midges were collected in a New Jersey type of light trap that connected with a cage rather than with a killing bottle; this trap was set up beside the duck pens. Ducks were injected with suspensions of black flies, mosquitoes, and biting midges after comminution in blood in a tissue grinder. These ducks were held either in the animal house at Algonquin Park in cages covered with copper screening having openings of 0.01 in. or, in most cases, in the animal house in Toronto where simuliids, biting midges, and mosquitoes were absent. Some of the insects that were known to have fed on ducks were dissected; others were fixed in Bles fluid, sectioned, and the resulting preparations were examined for developing stages of *Haemoproteus*.

Results

Many of the ducks that were placed out-of-doors, especially during June and July, died as a result of infection with *Leucocytozoon* before *Haemoproteus* was detected. *H. nettionis* developed in most of those that survived (Table I). The exact date on which many of the ducks acquired infection with *Haemoproteus* could not be determined with certainty since most of the ducks were left outside continuously from the dates shown in the table. Nevertheless, from infections observed in ducks 1561, 1608, 1609, 1713, 1746, and 1760 the prepatent period obviously may be as short as 14–17 days. The longer periods between exposure and the observation of parasites in the other ducks may result from a combination of factors. (i) It is unlikely that all ducks were infected on the first day of exposure. (ii) Blood smears were not always made on the first day of patency, otherwise parasites more than 2–5 μ in length would not have been present in the blood on the day it was examined for the first time. (iii) Small parasites may be overlooked on the first day of patency if the infection is light.

Infections with *H. nettionis* were never detected until late June or early July whereas *Leucocytozoon* was transmitted in late May and early June as well (6). The parasites in some ducks (1481, 1482, 1483, 1660, 1666) were relatively large on the first day that they were detected. Obviously the parasites in these ducks must have been present for some time previously, in some instances for a few days only and in others possibly for a few weeks, as shown by the dates of previous blood smears. *H. nettionis* was detected at an earlier date in 1955 than in 1954 and 1956. Since the prepatent period may be as short as 2 to 3 weeks, transmission of *nettionis* must have begun about mid-June in 1954 and 1956 and about June 8 in 1955. The vector must have been present some time in advance of these dates to allow for a cycle of sporogony in a sufficient number of vectors after they had fed on infected ducks. Infection was not detected in ducks 1783, 1784, and 1786 that were outside from May 15 to June 15, 1956, but it appeared in ducks 1792 and 1797, which were outside continuously from June 5 and 12 respectively. This suggests that transmission began on or after June 15 in 1956. This is indicated also by the fact that infection developed in ducks 1805, 1807, and 1810, which were exposed from the evening of June 14 to June 17 only. Transmission of *Leucocytozoon* began much sooner, for infection with *L. simondi* was detected in ducks 1783, 1784, 1786, and 1792 on June 11 and in all of the above ducks by June 29. Transmission of *H. nettionis* occurred, obviously, in July each year and only to a limited extent in August.

No data on the relative abundance of biting insects that might conceivably be vectors of *H. nettionis* were available for 1954. The weather in May 1954 and 1956 was colder than in May 1955 and this, by limiting the number of blood-sucking insects that were on the wing, would account for the later commencement of transmission in 1954 and 1956. In 1955 many simuliids of the subgenus *Eusimulium* fed on ducks in the latter part of May and early June, and *S. rugglesi* fed commonly on them in June and early July.

TABLE I

RESULTS OF EXAMINATION OF BLOOD OF DUCKS THAT ACQUIRED NATURAL INFECTIONS WITH *H. nettionis*

Year	Duck	Date of exposure*	Date parasites seen and date of previous blood smear		Days from exposure until parasites seen	Approx. length (μ) on first day observed	Remarks
1954†	1477	20/5–26/5	Negative		—		Size of parasites indicates that some must have been present 1 to several days prior to the date on which they were noticed first. No data on the relative abundance of various biting insects were available for this year.
	1478	20/5–26/5	Negative		—		
	1479	20/5–26/5	Negative		—		
	1481	29/5	9/7	23/6	41	28	
	1482	29/5	9/7	23/6	41	21	
	1484	29/5	9/7	23/6	41	21	
	1491	4/6	9/7	23/6	35	6	
	1496	4/6	9/7	29/6	35	21	
	1508	15/6–17/6	15/7	26/6	30	14	
	1561	25/6–26/6	9/7	8/7	14	< 5	
	1580	9/7–11/7	7/8	15/7	29	28	
	1534	9/7	2/8	23/7	24	12	
	1608	16/7–20/7	3/8	2/8	18	< 5	
	1609	16/7–20/7	3/8	3/8	18	< 5	
	1615	1/8	28/8	22/8	27	12	
1955‡	1660	9/5	29/6	28/5	51	21	Species of *Eusimulium* were common and feeding on ducks in May and early June. *S. rugglesi* was abundant and feeding on ducks in June and early July. Several species of mosquitoes and anthropophilic biting midges were common in June and early July. No search was made for ornithophilic species of biting midges or mosquitoes.
	1664	18/5	29/6	27/5	42	5	
	1666	18/5	29/6	28/5	42	21	
	1674	29/5	29/6	6/6	31	5	
	1676	29/5	29/6	8/6	31	21	
	1682	4/6	29/6	21/6	25	21	
	1685	4/6	29/6	16/6	25	12	
	1686	4/6	29/6	16/6	25	5	
	1687	4/6	29/6	16/6	25	12	
	1692	4/6	10/7	16/6	36	12	
	1693	4/6	29/6	16/6	25	5	
	1694	4/6	29/6	16/6	25	21	
	1695	4/6	29/6	16/6	25	6	
	1703	14/6	7/7	26/6	23	28	
	1706	14/6	7/7	29/6	23	< 5	
	1707	14/6	1/7	30/6	17	< 5	
	1708	14/6	30/6	29/6	16	< 5	
	1711	14/6	7/7	26/6	23	5	
	1712	14/6	7/7	26/6	23	14	
	1713	14/6	30/6	29/6	16	5	
	1733	26/6	25/7	19/7	29	6	

	1735	26/6	19/7	16/7	23	6	
	1737	26/6	25/7	19/7	29	14	
	1739	26/6	26/7	19/7	30	28	
	1740	26/6	16/7	12/7	20	14	
	1741	26/6	16/7	12/7	20	21	
	1743	2/7	24/7	17/7	22	21	
	1744	2/7	24/7	17/7	22	21	
	1745	2/7	24/7	17/7	22	21	
	1746	2/7	17/7	15/7	15	5	
	1747	2/7	24/7	17/7	22	14	
	1752	2/7	24/7	17/7	22	5	
	1758	9/7	6/8	30/7	28	6	
	1759	9/7	6/8	30/7	28	5	
	1760	9/7	25/7	24/7	16	5	
	1761	9/7	6/8	30/7	28	14	
	1762	9/7	8/8	30/7	30	5	
	1763	9/7	30/7	28/7	22	6	
	1770	17/7	7/8	1/8	21	21	
	1774	25/7	22/8	15/8	28	14	
1956	1783	15/5–15/6	Negative				Species of *Eusimulium* were feeding on ducks during the last week of May and first two weeks of June. *S. rugglesi* was taken from ducks from June 11 until late in August. It was especially abundant during the latter part of June and July. Mosquitoes that had fed on ducks were collected from early June until late August. Biting midges that fed on ducks were first collected on June 8. They were especially abundant during the last week of June and early July. Fewer were taken in the latter part of July and almost none in August.
	1784	15/5–15/6	Negative				
	1786	15/5–15/6	Negative				
	1792	5/6	13/7	2/7	38	5	
	1797	12/6	13/7	30/6	31	5	
	1805	14/6–17/6	3/7	27/6	19	< 5	
	1807	14/6–17/6	3/7	27/6	19	< 5	
	1810	14/6–17/6	9/7	9/7	25	6	
	1827	19/6	13/7	6/7	24	5	
	1834	23/6	13/7	4/7	20	< 5	
	1838	23/6	13/7	2/7	20	5	
	1840	23/6	13/7	2/7	20	5	
	1842	23/6	13/7	2/7	20	5	
	1843	23/6	13/7	2/7	20	< 5	
	1845	24/6	13/7	2/7	19	5	
	1851	24/6	13/7	2/7	19	12	
	1862	30/6	18/7	17/7	18	5	
	1863	30/6	20/7	18/7	20	5	
	1866	30/6	20/7	13/7	20	28	
	1877	3/7	26/7	22/7	23	< 5	
	1922	28/7	19/8	16/8	22	6	
	1924	28/7	19/8	16/8	22	14	

*Exposed from date shown until beginning of September unless indicated otherwise.
†Records are limited for this year as some ducks were not kept, or did not survive, long enough for *Haemoproteus* to appear.
‡*Haemoproteus* was not observed in three and four ducks exposed continuously for 1 month beginning July 25 and Aug. 2, respectively.

Mosquitoes were common also in June and July and some fed on ducks although few did so compared to the number of *S. rugglesi*. Anthropophilic biting midges were abundant in June and early July but no search was made for ornithophilic species. Hippoboscids were never recovered from any of the ducks. It seemed probable that the vector of *H. nettionis* might be some simuliid, mosquito, or biting midge. Consequently in 1956 an intensive effort was made to discover the insects that were feeding on the ducks when they became infected with *Haemoproteus*. The suitability of these insects as intermediate hosts was tested by injecting them into ducks and by examining sections of insects that had fed on infected ducks.

In 1956 species of *Eusimulium* were feeding on ducks in late May and early June (Table I) but transmission of *H. nettionis* apparently did not begin until about mid-June. These simuliids were presumably not responsible for the transmission. *S. rugglesi* was first taken on June 11 and was abundant by June 23 and many of this species were feeding on ducks. Specimens of *S. rugglesi*, known to have fed on ducks infected with *H. nettionis*, were kept in captivity for 7 to 13 days and then comminuted in blood and injected into six ducks. None of them became infected with *Haemoproteus*.

The possibility remained that some biting midge or mosquito is the intermediate host. An experiment was designed, therefore, to determine whether transmission was taking place by day or night. Six ducks were exposed from 9 p.m. to 3 a.m. E.S.T. for 7 days beginning July 23 and then from 9 p.m. until 12 midnight for another 3 weeks. Two of these ducks died from an unknown cause within 2 weeks after they were exposed for the first time. The remaining four had gametocytes of *H. nettionis* in the peripheral circulation less than 4 weeks after they were exposed. None were infected with *Leucocytozoon*. Six controls, exposed continuously beginning July 24, became infected with *Leucocytozoon* and died within 2 weeks. A second control group of five ducks was exposed continuously after July 28. All became infected with *Leucocytozoon*. Two of these ducks survived for more than 2 weeks and these were infected with *Haemoproteus* also. Obviously, *Haemoproteus* was being transmitted to the ducks during the night, most probably by biting midges or mosquitoes. Observations after dark with the aid of a flashlight revealed that many biting midges and some mosquitoes fed on the ducks. Midges were taken in the light trap for the first time on June 8 although some were undoubtedly present for a few days prior to this. Many of these midges had fed on ducks; moreover most of those taken in the light trap were a species that did not appear to feed on man. The ornithophilic and nocturnal habits of these midges are of interest and explain in part why their role as intermediate host for *H. nettionis* was not discovered previously. These biting midges were especially abundant during the last week in June and the first 2 weeks of July. Some mosquitoes, although few in number compared to the midges, were also feeding on ducks from early in June until late in August. These various observations led to the conviction that a species of mosquito or biting midge is the intermediate host of *H. nettionis*.

It seemed more likely to be a biting midge as hundreds of them were feeding on ducks compared to a few score of mosquitoes. Moreover, biting midges were most abundant when transmission of *H. nettionis* was occurring.

These possibilities were tested in experiments in which specimens of mosquitoes and biting midges, following comminution in blood, were injected into ducks. In addition, some specimens, known to have fed on ducks infected with *Haemoproteus*, were dissected and others were fixed and sectioned. Two species of mosquitoes (*Mansonia perturbans* and *Anopheles earlei*) were known to feed on ducks during the time when transmission of *Haemoproteus* was occurring. Some of these mosquitoes were captured after they had fed on ducks infected with *Haemoproteus* and, after they had been held in captivity for various periods, were comminuted in blood and injected into ducks. In this way four ducks received injections of 5 to 15 mosquitoes that had ingested gametocytes of *H. nettionis* 11, 13, 14, and 21 days previously. No infections with *Haemoproteus* resulted from these injections. Moreover, neither oöcysts nor sporozoites were found in any of the mosquitoes that were dissected or sectioned.

Likewise the possibility of biting midges being intermediate hosts was investigated. Specimens of biting midges that were collected in the light trap on successive nights were comminuted in blood and the suspensions were injected intraperitoneally into ducks; the results are shown in Table II. *H. nettionis* developed in seven of the nine ducks that were injected with 150 or more of the biting midges that were captured in the light trap. Conceivably more than one species of biting midge was included in each of the suspensions although one species of *Culicoides* was much more abundant than any of the other five that were taken in this way. This species was identified as *Culicoides*, near *piliferus*, by Mr. J. A. Downes, Entomology Division, Department of Agriculture, Ottawa. He will describe it later. This species was known to feed on ducks and was the only one taken from ducks in a cage placed over the birds as described previously. Further evidence that this species is the intermediate host of *H. nettionis* was obtained from a second experiment (Table II) in which specimens of *Culicoides*, known to have fed previously on ducks with *Haemoproteus*, were injected into other ducks. Three ducks were injected with suspensions of the insects that ingested gametocytes of *H. nettionis* 4, 10, and 11 days previously. *Haemoproteus* developed in the two latter ducks, thus confirming the results obtained from the other injections and indicating that a biting midge is a suitable intermediate host for *H. nettionis*.

A comparison of the largest parasites that were observed on successive days of patency gives an indication of the minimum time required for parasites to reach their full size. This assumes, of course, that the small parasites that are seen on the first day of patency grow into the large gametocytes that are easily recognized subsequently. An estimate of the rate of growth of gametocytes is possible from a scrutiny of their size in ducks 1917–1919 on different days of patency (Table III). The smallest parasites to be detected

TABLE II

SUMMARY OF INFECTIONS WITH *H. nettionis* PRODUCED IN 1956 BY INJECTING DUCKS WITH SUSPENSIONS OF BITING MIDGES

Duck	Date of injection	No. midges injected	Date parasites seen and date of previous smear		Prepatent period, days	Remarks
1860	30/6	500	20/7	13/7	< 21	Some parasites 12 μ in length
1875	2/7	150	Negative			
1887	12/7	200	Negative			
1903	20/7	500	7/8	3/8	< 18	Some parasites 12 μ in length
1904	20/7	1000	7/8	3/8	< 18	Some parasites 10 μ in length
1917	25/7	400	10/8	—	16	Parasites less than 5 μ
1918	26/7	220	10/8	—	15	Parasites less than 5 μ
1919	27/7	250	10/8	—	14	Parasites less than 5 μ
1925	28/7, 1/8, 3/8, 8/8	800	23/8	13/8	?	Some parasites 12 μ in length
1905	23/7	10	Negative			Midges had fed 4 days previously on infected ducks
1926	30/7	36	20/8	17/8	21	Midges had fed 11 days previously on infected ducks
1928	30/7	40	13/8	—	14	Midges had fed 10 days previously on infected ducks

TABLE III

RATE OF GROWTH OF *H. nelsonis* AS DETERMINED BY LENGTHS (IN μ) OF PARASITES ON VARIOUS DAYS BEGINNING WITH THE DAY THEY WERE DETECTED IN DUCKS INFECTED ARTIFICIALLY BY INJECTIONS OF SUSPENSIONS OF BITING MIDGES

		Duck No.				
		1903*	1904*	1917	1918	1919
Aug. 7	Max.	12	12			
	Min.	3	1			
	Av.	6.8 (50)†	6.3 (50)			
Aug. 8	Max.	17	18			
	Min.	1	1			
	Av.	8.9 (50)	9.4 (50)			
Aug. 9	Max.	28	26			
	Min.	1	1			
	Av.	7.8 (48)	10.3 (50)			
Aug. 10	Max.			4	3	3
	Min.			1	1	1
	Av.			2.6 (50)	1.9 (22)	1.6 (20)
Aug. 13	Max.			15	10	12
	Min.			4	2	2
	Av.			7.3 (50)	5.8 (50)	5.1 (50)
Aug. 14	Max.			21	13	14
	Min.			4	3	3
	Av.			9.6 (50)	7.9 (50)	4.9 (50)
Aug. 16	Max.			28	26	26
	Min.			3	2	3
	Av.			15.4 (42)	12.6 (50)	13.8 (50)

*First smears made 18 days after duck injected. Size of parasite indicates that some were present prior to August 7.

†Number of parasites measured is given in parentheses after the average.

in smears of peripheral blood measure from 1 to 2 μ. Those that were 4 μ in length on the first day that they were detected in ducks 1917–1919 obviously must have been in the red cells for some hours previously. The size of the parasites observed on succeeding days reveals that 4 to 6 days are required for a parasite to reach the length of 28 μ, by which time it surrounds completely the nucleus of the red blood cell. Unfortunately, blood smears were not prepared from ducks 1903 and 1904 between August 3 and August 8 so that when parasites were detected on August 8 some, obviously, were partially grown. Nevertheless, an additional 2 days elapsed before any were found that filled the red blood cells.

Knowledge of the rate of growth of the gametocytes can be of assistance in determining the prepatent periods. If large or partially grown parasites are present on the first day that infection is detected, it is reasonable to assume that they were present for some time previously. Consequently, when the results given in Table II are considered along with the rate of growth of the parasites given in Table III, it can be concluded that the prepatent period for

H. nettionis, although sometimes 21 days, is more often nearer 14 days. The first day of patency was overlooked, possibly, in some instances, owing to the scarcity of parasites in the relatively light infections (A and B of Herman and Glading's (10) classification) that occurred in many ducks.

A detailed account of sporogony in *Culicoides* awaits further investigation and only brief reference to some of the stages is included here. Oökinetes observed in stained smears of the stomach contents of a midge 36 hours after it ingested gametocytes were long, slender, lacking pigment, and highly vacuolated (Fig. 1). The absence of pigment in the oökinete in other species was noted by the Sergents (16), Mezincescu (14), Gonder (7), and Wasielewski and Wülker (18) although Adie (1) reported pigment in the oöcyst of *H. columbae*. Structures 10–12 μ in diameter and regarded as developing oöcysts (Fig. 2) have been found in the stomach wall in sections of a specimen of *Culicoides* that was fixed in Bles fluid about 4 days after the insect had fed on an infected duck. Other stuctures, that are regarded as sporozoites (Fig. 3), were seen in sections of salivary glands of specimens of *Culicoides* that were fixed 10 days after the insects had ingested gametocytes. These sporozoites seem to be longer and more slender than those of *Leucocytozoon* but their exact size and appearance awaits study in fresh preparations and in stained smears of the contents of the salivary glands of infected *Culicoides*.

Discussion

The discovery of *Culicoides* as an intermediate host for *Haemoproteus* raises interesting questions. Will *H. nettionis* also develop in hippoboscids, if any species can be found that will feed on ducks? Will other species of *Haemoproteus* develop in biting midges as well as the species of hippoboscids that have been shown to be vectors? If so, will these midges have nocturnal and ornithophilic habits as found in the present study? Unless birds are kept in finely screened cages or in an area where midges do not occur, transmission could take place without *Culicoides* being suspected as a vector. Clearly from the observations and experience of this past summer it is desirable in the area under study to conduct future work on natural transmission at night rather than during the day. If biting midges should prove to be hosts for other species of *Haemoproteus* it lends support to the view, expressed by Manwell (13), that *Haemoproteus* is perhaps more primitive than either of the two closely related genera *Leucocytozoon* and *Plasmodium*.

A prepatent period of 14 days is considerably shorter than that reported for species of *Haemoproteus* which have been transmitted by hippoboscids, although Herman and Bischoff (9) and Tarshis (17) observed parasites in some of their California valley quail 21 and 20 days respectively after the birds were inoculated with sporozoites or hippoboscid flies were placed on them. The size of the parasite when first seen in the blood is not indicated in most reports. If the parasites were partially or fully grown when first seen and if their rate of growth resembles that observed for *H. nettionis* then the prepatent period is less than the 3, 4, or more weeks that others report.

PLATE I

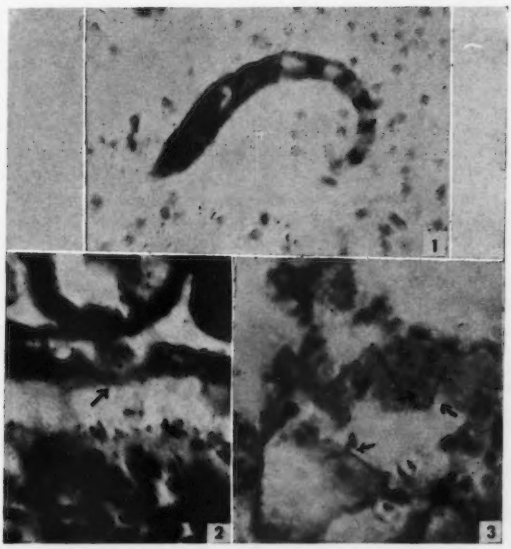

FIG. 1. Oökinete of *Haemoproteus nettionis* from smear of stomach content of *Culicoides* sp. 36 hours after the midge had fed on an infected duck. Approx. 1200×

FIG. 2. Section through stomach wall of *Culicoides* sp. that fed 3–4 days previously on duck infected with *H. nettionis*; shows structure regarded as a developing oöcyst on the outer wall of the stomach. Approx. 1000×

FIG. 3. Section through salivary gland of *Culicoides* sp. that fed 10 days previously on duck infected with *H. nettionis* shows structures that are regarded as sporozoites. Approx. 1000×

Acknowledgments

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THE METAZOAN PARASITES OF THE HETEROSOMATA OF THE GULF OF ST. LAWRENCE

I. ECHINORHYNCHUS LAURENTIANUS SP. NOV. (ACANTHOCEPHALA: ECHINORHYNCHIDAE)[1]

KEITH RONALD

Abstract

Echinorhynchus laurentianus sp. nov. (Acanthocephala: Echinorhynchidae) is described from *Hippoglossoides platessoides*, *Hippoglossus hippoglossus*, *Pseudopleuronectes americanus*, and *Scophthalmus aquosus* from the Gulf of St. Lawrence.

The common acanthocephalan parasite of the Heterosomata of the northwest Atlantic is *Echinorhynchus gadi* Müller, 1776 and the author has often found it in the presence of the new species described below. Cursory examination revealed no morphological differences between the two parasites, the principal criterion for distinguishing them lying in the hook count. *E. gadi* has 18–22 longitudinal rows of hooks on the proboscis thus differing radically from the new species, which has 14–16 rows.

Echinorhynchus ***laurentianus*** sp. nov.

The body of the living worm is pink or white in color. Sexual dimorphism is fairly well marked, the female being the larger.

Female.—Length 10–15 mm., width 0.80–1.40 mm. Body usually curved ventrally giving the proboscis the appearance of extruding from the ventral surface. Length of extended proboscis 0.35–0.65 mm., width 0.11–0.15 mm. The proboscis bears 14–16 longitudinal rows of hooks set in 11–13 transverse rows. Larger hooks measure 0.038–0.048 mm. in blade (thorn) length, 0.020–0.025 mm. in height. Root of hook 0.024–0.029 mm. long. Hooks curved and conical in shape except the smaller proximal ones, which are triangular. Lemnisci broadly elongated measuring 0.80–1.10 mm. in length and 0.18–0.32 mm. in width, one lying on each side of the proboscis sac. Proboscis sac, 0.55–0.70 mm. long and 0.32–0.40 mm. wide, slightly shorter than lemnisci and more tubular in shape. Neural ganglion situated posteriorly in proboscis sac with two retinacula passing backwards from posterior extremity of ganglion, 1 mm. long; root of dorsal retractor triradiate, that of ventral retractor weakly biradiate. In the juvenile worm the ovarian balls almost fill the entire body cavity. The ova, when formed, have one end pointed, the other stalked, terminating in a knob.

Male.—Shorter than female and usually less curved; 5–11 mm. long, 0.55–0.72 mm. wide. Proboscis resembles that of female both in shape and armature. Testes tandem, bluntly elliptical in shape, 0.60–0.95 mm. long

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Contribution No. 57 from Département des Pêcheries, Marine Biological Station, Grande-Rivière, Qué., and the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada.

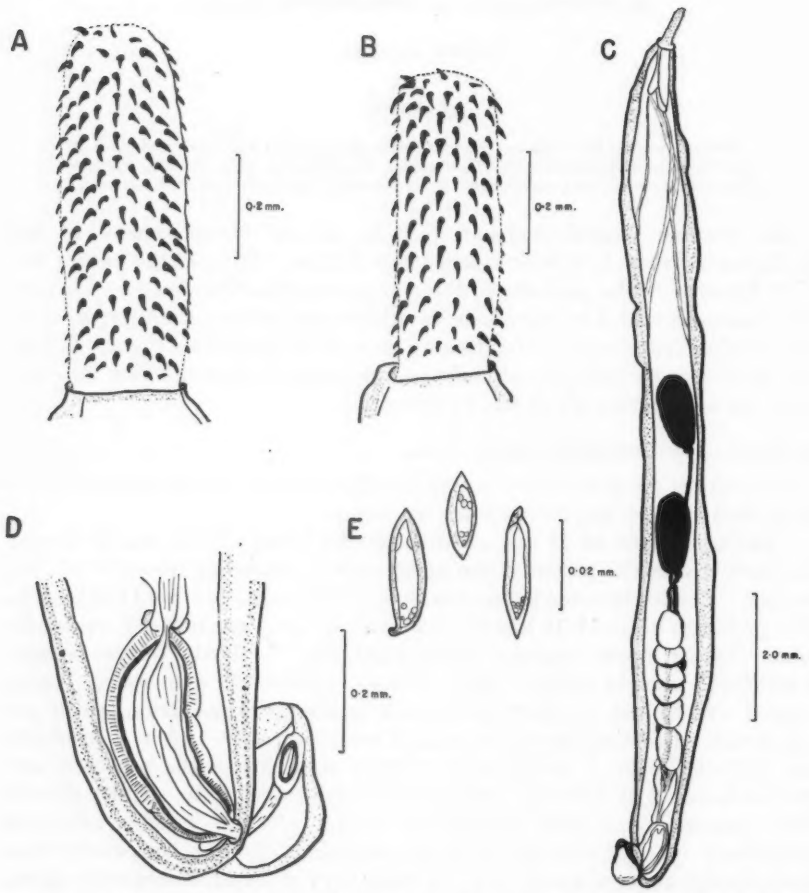

FIG. 1. (A) Proboscis of *Echinorhynchus gadi*. (B) Proboscis of *E. laurentianus*. (C) Male *E. laurentianus*. (D) Partly extruded copulatory bursa of *E. laurentianus*. (E) Ova of *E. laurentianus*.

and 0.28–0.35 mm. wide. Testicular ligament present between the two testes. Sperm ducts leave posterior end of each testis, twist together, and join before entering urogenital canal pouch. Six cement glands, intermixed and superimposed, roughly globular in shape. Cement ducts empty into the common sperm duct. Copulatory bursa bean-shaped when everted, closely folded within bursa capsule when introverted. Gonopore situated dorsomedially and poorly defined when copulatory bursa introverted.

Hosts: *Hippoglossoides platessoides* (Fabricius, 1780),
Hippoglossus hippoglossus (Linné, 1758),
Pseudopleuronectes americanus (Walbaum, 1792),
Scophthalmus aquosus (Mitchill, 1815).

Location: Digestive tract.

Locality: Gulf of St. Lawrence.

Incidence: *E. laurentianus* sp. nov. was present in 46% of the 400 fish examined. Sixty-one per cent of the worms were females, 39% males, and 29% immatures of both sexes.

DELTOKERAS SYNALLAXIS SP. NOV. (DILEPIDIDAE) FROM SYNALLAXIS RUTILANS TEMM.[1]

JUNE MAHON[2]

Abstract

Deltokeras synallaxis sp. nov. (Dilepididae) from a passeriform bird from Brazil is described and the taxonomic status of the genus is examined.

A vial containing portions of a single tapeworm was forwarded to the Institute of Parasitology by Dr. Ernst Mayr, Museum of Comparative Zoology, Harvard College, who received the material from Dr. Helmut Sick, of the Fundação Brasil Central. The specimen proved to be a new species. The drawings were made with the aid of a camera lucida.

Deltokeras synallaxis sp. nov.

Dilepididae Fuhrmann, 1907, Paruterininae Fuhrmann, 1907

Host: *Synallaxis rutilans* Temm. (Passeriformes).
Locality: Brazil.
Type specimen: U.S. National Museum, Helminthological Collection, Washington, D.C.

The worm is 25 mm. long with a maximum breadth of 1 mm. Most of the segments are broader than long, only the most gravid ones being longer than they are broad. The anterior part of the strobila is near-cylindrical in cross-section. The genital pores are irregularly alternating.

The scolex, mounted in Canada balsam, has a diameter of 336 by 416 μ as viewed head on (Fig. 1), and is provided with four suckers, 95 to 117 μ by 124 to 131 μ. The rostellum, 95 to 102 μ in diameter, is armed with 38 triangular hooks arranged in a double crown (Fig. 2). The hooks were mounted in gum chloral. They are all of similar size, measuring 16 to 18 μ by 11 to 13 μ. The handle is bifid.

The excretory system is of the normal type, composed of a pair of narrow, longitudinal, dorsal vessels and a pair of wider, thin-walled vessels, joined by a posterior, transverse commissure in each segment.

Paired, longitudinal nerve trunks are situated lateral to the excretory vessels.

The muscular system is not strongly developed. The longitudinal, cortical musculature consists of two layers. The outer one is composed of numerous single or paired muscle fibers, and the inner layer of about 24 small bundles, each of which is composed of from four to five fibers (Fig. 3).

The six to eight testes (Fig. 4) are situated posteriorly in the segment.

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Contribution from the Institute of Parasitology, McGill University, Macdonald College P.O., Que., Canada, with financial assistance from the National Research Council of Canada.
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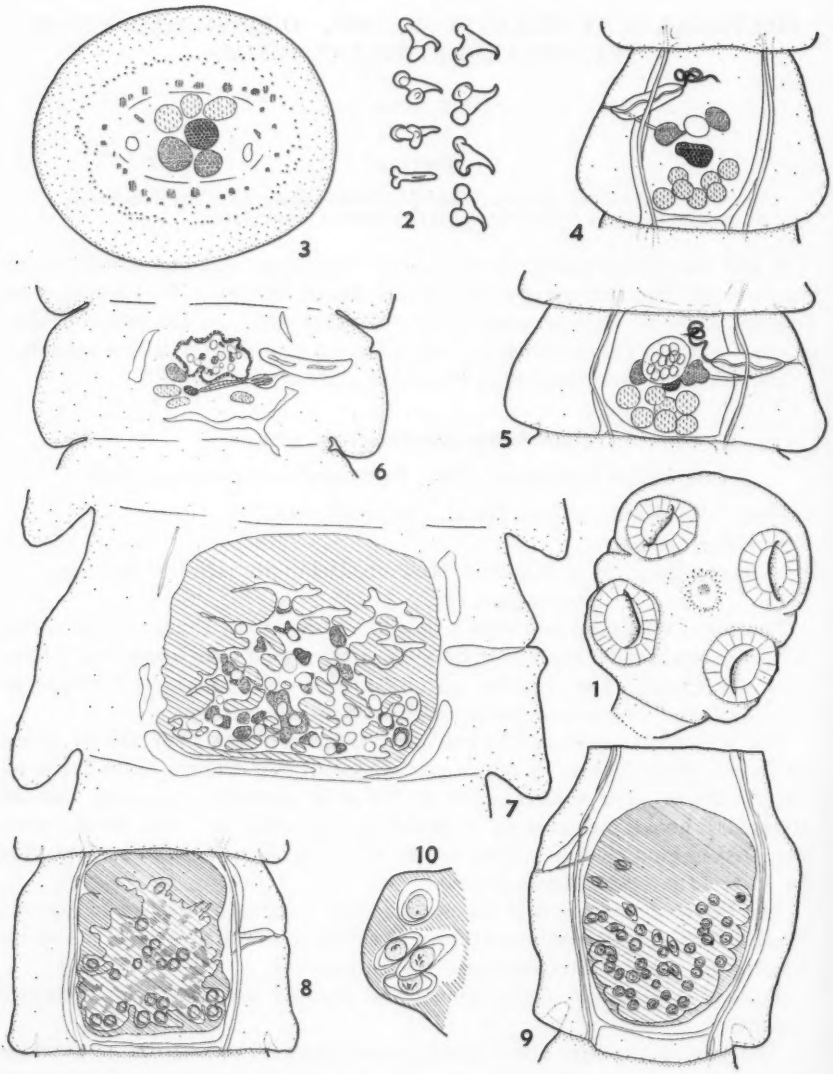

FIGS. 1 to 10. *Deltokeras synallaxis.*
FIG. 1. En face view of scolex. FIG. 2. Rostellar hooks in various positions. FIG. 3. Transverse section of mature segment. FIG. 4. Dorsal view of mature segment. FIG. 5. Dorsal view of segment showing young uterus. FIG. 6. Longitudinal section showing young uterus. FIG. 7. Longitudinal section showing further development of uterus. FIG. 8. Whole mount showing formation of uterine capsules. FIG. 9. Whole mount showing gravid segment. FIG. 10. Ripe eggs enclosed in a portion of the paruterine organ.

The vas deferens is slightly convoluted before it enters the cirrus pouch. There is neither an external nor an internal seminal vesicle. The cirrus pouch, 85 to 110 μ long and 18 to 22 μ in maximum width, extends just past the poral excretory vessels. It contains the lightly coiled, unarmed cirrus, and opens into a shallow non-muscular genital atrium, which is situated at the anterior third of the lateral margin of the proglottis.

The straight, thin-walled vagina opens from the genital atrium ventral to the cirrus pouch. It then passes posterior to the latter and both genital ducts pass between the poral excretory canals. In fully mature segments a small, oval receptaculum seminis is present. The centrally situated ovary is bilobed and contains a small number of large, conspicuous ova. At the junction of the two lobes arises the oviduct, into which leads the receptaculum seminis and the vitelline duct. The vitelline gland is compact, situated behind and somewhat ventrally to the ovary. It is composed of large, darkly staining, granular cells smaller, however, than those of the ovary. The Mehlis' gland was not observed.

The uterus appears as a small, spherical sac between and just anterior to the ovarian lobes (Figs. 4, 5). In sectioned material dense, darkly-staining, fibrous tissue may be seen arising from the parenchyma to closely surround the uterus (Fig. 6). As it increases in size the uterus becomes lobed. Concurrent with the evolution of the uterus the dense paruterine tissue continues to develop, accompanying the uterine walls between the lobes of the uterus (Fig. 7). The uterus eventually occupies the whole of the medulla but never extends laterally beyond the excretory vessels. Finally, each of the small number of eggs becomes enclosed in a portion of the uterus, that is, within a uterine capsule, and the interstices between the capsules are filled with paruterine tissue (Fig. 8). In the fully gravid segment (Fig. 9) the majority of the eggs are grouped posteriorly, the rest of the medulla being occupied by the paruterine organ.

In the most gravid segments the egg shells become lemon-shaped (Fig. 10). Viewed in profile, they measure 55 to 65 μ by 24 to 29 μ, and the embryo measures 33 to 36 μ by 22 to 26 μ. Viewed along the longitudinal axis, the eggs have a diameter of 26 to 29 μ.

Discussion

The species so far described for the genus *Deltokeras* are *D. ornitheios* Meggitt, 1927, the type species; *D. campylometra* Joyeux and Baer, 1928; *D. delachauxi* Hsü, 1935; *D. multilobatus* Olsen, 1939; and *D. granatensis* Lopez-Neyra, 1943. These species are all recorded from passeriform birds. Their main diagnostic features are listed in the table.

D. synallaxis differs from all but *D. delachauxi* in having irregularly alternating genital pores, and it differs from the latter in the number of testes, size of the cirrus pouch, and to a lesser extent the size and shape of the rostellar hooks. It differs from all other species in the number of testes and the size of the cirrus pouch, and from all but *D. multilobatus* in the size of the hooks.

TABLE I

MEASUREMENTS OF *Deltokeras* SPECIES

	D. ornitheios Meggitt, 1927	*D. campylometra* Joyeux & Baer, 1928	*D. delachauxi* Hsü, 1935	*D. granatensis* Lopez-Neyra, 1943	*D. multilobatus* Olsen, 1939	*D. synallaxis* sp. nov.
	Described by:					
	Meggitt, 1927	Joyeux & Baer, 1928	Hsü, 1935	Lopez-Neyra, 1943	Olsen, 1939	Personal
Length	40 mm.	20 mm.	68 mm.	15.5–18 mm.	45 mm.	25 mm.
Breadth	1.1 mm.	1.3 mm.	1.06 mm.	0.48 mm.	0.6–0.7 mm.	1 mm.
Scolex	—	500–570 μ	256 μ	530–550 μ	243–324 μ	336 × 416 μ (*en face* view)
Suckers	—	150–200 μ	—	160–120 μ	87–102 μ	95–117 × 124–131 μ
Rostellum	—	50–70 μ	90 × 50 μ	105 μ	72–91 μ	95–102 μ
No. hooks	80	46	6 (?)	100	Only 2	38
Hook length	27–31 μ (2 rows)	10–15 μ	14–15 × 11–12 μ	20–22 and 18–20 × 12–15 μ	17–19 μ	16–18 × 11–13 μ
Testes	20	20 (25–30)	15–17	18–22	12–16	6–8
Cirrus pouch	140–200 × 40 μ	250 × 40 μ	108–120 × 43–39 μ	100–170 × 30–45 μ	121–133 × 19–23 μ	85–110 × 18–22 μ
Genital pore	Unilateral	Unilateral	Irregularly alternating	Unilateral	Unilateral	Irregularly alternating
Eggs	None	70 × 45 μ	56 × 40 μ	55–60 × 42–50 μ	53–57 × 30 μ (in section)	55–65 × 24–29 μ
Embryos	—	40 × 25 μ	—	22–28 × 29–30 μ	30–31 × 23–27 μ	33–36 × 22–26 μ
Host	*Urocissa occipitalis* (Bl.)	*Pyromelana franciscans* Is. *Penthetriopsis macrura* Gm. *Potamorhynchus remigialis* Finsch-Hartl.	*Lanius schach schach* L.	*Lanius c. collurio* L.	*Seleucides m. melanoleucos* (Daud.)	*Synallaxis rutilans* Temm.
Locality	Rangoon	Africa	China	Granada	New York Zoo	Brazil

NOTE: Abbreviations used in table: breadth: maximum breadth of specimens; cirrus pouch: dimensions of cirrus pouch; eggs: diameter of eggs; embryo: diameter of embryo; length: maximum length of specimens; no.: number; testes: number of testes per segment.

The latter is characterized by a deeply lobed ovary with eight or nine divisions. The lemon-shaped eggs have not been described for the other species, which may be due to the absence of sufficiently gravid segments. One hesitates to consider this type of egg as a specific character.

The systematic position of *Deltokeras* has been the subject of some confusion. The genus has been variously placed in two families and four different subfamilies, and it would seem opportune to give a brief survey of the history of this genus.

The genus *Deltokeras* was erected by Meggitt (6) in 1927 with *D. ornitheios* from *Urocissa occipitalis* (Bl.) from the Victoria Memorial Park, Rangoon, as type species. Meggitt was able to observe only the early development of the uterus as there were no gravid segments in his specimens. As the diagnosis of the genus he gives:

> "Rostellum armed with triangular hooks; genital pores unilateral; testes numerous, posterior and lateral to the female glands; uterus persistent. Type species: *D. ornitheios*."

Lack of information on the development of the uterus was the first cause of confusion, Meggitt believing that the uterus was persistent and that a paruterine organ was lacking. He proposed the erection of a new family, Biuterinidae, characterized by triangular rostellar hooks, and the division of this family into two subfamilies, Biuterininae (type genus *Biuterina*, with the uterus replaced by a paruterine organ) and the Deltokerinae (type genus *Deltokeras*, with the uterus persistent).

In 1928, Joyeux and Baer (3) described *D. campylometra* from *Pyromelana franciscans* Is. and *Penthetriopsis macrura* Gm. from French West Africa. In parenthesis, they suggest that *Taenia* sp. described by Klaptocz in 1908 from *Potamorhynchus remigialis* Finsch-Hartl from the region of the White Nile is identical with *D. campylometra*. These authors have specimens sufficiently gravid to observe the dense fibrous tissue surrounding the uterus, but not ripe enough to observe the full development of a paruterine organ. They consider that the erection by Meggitt of a new family, the Biuterinidae, based exclusively on the shape of the rostellar hooks, is unnecessary, and they place the genus *Deltokeras* in the subfamily Dilepidinae of the family Dilepididae, with the remark that if a paruterine organ were, in fact, present then the genus would fall into the subfamily Paruterininae.

Fuhrmann (1) placed *Deltokeras* in the subfamily Paruterininae, and modified the generic diagnosis by adding: "the paruterine organ appears late and surrounds the uterus."

Hsü (2) described a new species, *D. delachauxi*, from *Lanius schach schach* L. from China. This was the first time that it was possible to follow the complete development of the uterus in ripe segments, and Hsü was able to observe the breakdown of the uterus into uterine capsules, and the formation of a distinct paruterine organ. He includes the genus in the subfamily Paruterininae, and further modifies the generic diagnosis to include: genital pores unilateral or irregularly alternating, uterus breaks down into egg

capsules, a paruterine tissue surrounds the uterus, later enclosing the egg capsules.

D. multilobatus is described by Olsen (7) from *Seleucides melanoleucos melanoleucos* (Daud.) from the New York Zoological Park, and he too places the genus in the subfamily Paruterininae. Olsen considers *D. multilobatus*, in which the paruterine organ is poorly developed, to be intermediate between *D. delachauxi* and *D. campylometra* on the one hand, in which the paruterine organ is well defined, and *D. ornitheios* on the other, in which the paruterine organ is absent. He suggests that if Meggitt's material were mature enough to show at least the early development of the uterus, then there would have been some indication of the paruterine organ, had the latter been present. However, the formation of the dense, fibrous tissue surrounding the young uterus can only be seen clearly in sectioned material and is not at all apparent in whole mounts. It would be surprising to find that a paruterine organ was present in some members of the species of one genus and absent in other members of the same genus.

In 1943 Lopez-Neyra (5) describes *D. granatensis* from *Lanius collurio collurio* L. from Spain. He is able to observe the formation of uterine capsules each containing one egg. He takes the view that a true paruterine organ is lacking, and that its function is carried out by the medullary parenchyma. Consequently, he does not admit of the inclusion of *Deltokeras* in the Paruterininae, but places it in the Monopylidiinae Lopez-Neyra, 1934, a subfamily of the Dilepididae. This subfamily was erected by Lopez-Neyra to accommodate the genus *Monopylidium* Fuhrmann, 1896, with *M. musculosum* Fuhrm., 1896 as the type species. The Monopylidiinae are characterized by having a uterus which breaks down into egg capsules and which becomes surrounded by a tissue of parenchymatous origin, which functions as a diffuse paruterine organ. Fuhrmann (1), however, considers *M. musculosum* to be in reality *Choanotaenia musculosum*, and the genus *Monopylidium* to be a synonym of *Choanotaenia*. This species is redescribed by Joyeux and Timon-David (4) and there is no mention of a paruterine organ.

Wardle and McLeod (8) place the genus in the family Biuterinidae.

With regard to the Biuterinidae, and as already remarked by Joyeux and Baer (3), there is no reason to erect a family whose sole distinguishing character is the shape of the rostellar hooks, and whose species fall into existing subfamilies in the Dilepididae. Also, Meggitt's subfamily Deltokerinae falls, the reason for separating the type genus from those in the Biuterininae no longer being valid.

The Paruterininae Fuhrmann, 1907 has the following diagnosis:

> "Uterus with a paruterine organ which receives the eggs in the ripe proglottids. Parasites of birds and mammals."

It is interesting to note that in the genera so far ascribed to this subfamily, the uterus is always persistent. At the moment there is no point in removing a single genus into a separate subfamily in which the uterus breaks down into egg capsules, and that is why the writer does not accept Lopez-Neyra's

subfamily, irrespective of any controversy over the position of the type species and about what the subfamily should be called.

Accordingly, the writer places this genus in the subfamily Paruterininae, and agrees with the generic diagnosis given by Hsü (2) as follows:

"Paruterininae: rostellum armed with two crowns of triangular hooks. Genital pores unilateral or irregularly alternating. Long cirrus pouch. Testes situated behind and to the sides of the ovary. Uterus breaks down into egg capsules. A paruterine tissue surrounds the uterus, later enclosing the egg capsules. Parasites of birds. Type species *D. ornitheios* Meggitt, 1927."

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PRELIMINARY OBSERVATIONS ON THE DIGESTIVE ENZYME SYSTEM OF THE BEAVER (CASTOR CANADENSIS)[1]

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Abstract

Results presented suggest that gastric digestion in the beaver is similar to that in other mammals. The more or less elaborate cardiogastric cellular system of the beaver seems to be concerned with the elaboration of pepsin and does not produce a mammalian cellulase. Some evidence of cellulolytic activity has been found in the caecal contents and is attributed to a commensal microflora. Pancreatic amylolytic activity of the beaver per unit body mass appears to be somewhat lower than in the pig.

Introduction

A knowledge of an animal's digestive processes is an essential prerequisite to a study of its nutritive requirements. The beaver's natural dietary and feeding habits have been the subject of extensive reviews (1, 9) which might lead one to suspect that this animal may possess a digestive enzyme system that is capable of hydrolyzing cellulose.

The digestive system of the beaver differs from that of other rodents in that it possesses on the lesser curvature of the stomach near the cardia, a more or less elaborate gland-like structure which has been referred to as the "cardiac gland" (8). Smith (8) suggested that the peptic activity of the secretion from this gland is less than that of the stomach proper. He also reported that both secretions may possess lipolytic and cellulolytic activity. Nasset (7) confirmed the presence of pepsin in the secretions of the "cardiac gland" but was unable to demonstrate cellulase activity. He noted that the parietal cells of the tubules of this gland are larger and two to three times more frequent than those of the stomach proper. This finding might imply greater peptic activity for the "cardiac gland" secretions.

The present report records certain findings obtained from experiments that were designed to clarify and extend those of Smith (8) and Nasset (7) and to provide basic information required for a study of beaver nutrition.

Methods and Materials

(*A*) *Animals*

Eight beavers were used in the present study. Four were captured on Vancouver Island within 48 hours of the initiation of the analytical procedures. The remaining four had been held in captivity for periods ranging from 1 week to 5 months.

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(*B*) *Dissecting Procedures*

After the animals had been sacrificed and the abdominal cavity exposed, hemostats were placed in pairs at the junction of the esophagus and stomach and at the pyloric sphincter. The cardiac and pyloric portions of the stomach contents were separated with a minium of admixing by placing a ligature around this section. The stomach was then removed and the separated contents of the pyloric and cardiac regions were recovered and filtered through several layers of cheesecloth. The two filtrates obtained were used for pH, free acid, and total acid determinations. The emptied stomach was then washed thoroughly with cold water. The mucous membrane was recovered from the muscularis mucosae and separate autolysates of the mucosae and the "cardiac gland" were prepared.

(*C*) *Analytical Procedures*

(*i*) *Total and free acids.*—The method of Töpfer's (6) was used for these determinations.

(*ii*) *Hydrogen-ion concentration.*—The Beckman (Model H) pH meter was used to determine active acidity of the undiluted gastric filtrates.

(*iii*) *Peptic activity.*—The method for preparing the autolysates and the quantitative estimation of proteolytic activity (Mett's procedure) were those given by Koch and Hanke (6). Ten concentrations (each in duplicate) of U.S.P. pepsin (Merck) served as standards. Three concentrations (all in duplicate) of the acidic glandular extracts were used as unknowns. Under the experimental conditions, using 3 mm. Mett's tubes, the U.S.P. pepsin produced 0.88 mm. of digestion in 24 hours. One unit of peptic activity has been taken to be the hydrolytic activity of 1 mg. of this U.S.P. pepsin.

(*iv*) *Pancreatic amylase activity.*—α-Amylase activity was determined using a slight modification of the Wöhlgenmuth procedure (4).

(*v*) *Cellulase activity.*—The presence or absence of cellulolytic activity was determined in the "cardiac gland" extract, stomach, and caecal contents by the method reported by Kitts and Underkofler (5).

Results and Discussion

The results of the various determinations are summarized in Table I. The pH range obtained is comparable with those recorded for man (2), the dog (2), and the horse (3). The values are in reasonably close agreement with those reported by Smith (8) and Nasset (7) for the beaver. It would be hazardous to comment on the significance of the free and total acid contents of the two filtrates. The range of values encountered is not unexpected in that it was difficult to control the amount and time of consumption of the ingesta present in the stomach at the time the animals were sacrificed. In the cardiac filtrates the total acid values ranged from between 0.28 to 0.44% with a mean of 0.35% expressed as hydrochloric acid. The corresponding range for the pyloric filtrates was 0.21 to 0.45% with a mean of 0.33%. Smith (8) and

TABLE I

FREE ACID CONTENT, TOTAL ACID CONTENT, pH, PEPTIC ACTIVITY, AND α-AMYLASE ACTIVITY OF THE BEAVER (*Castor canadensis*)

	Free acid content* of stomach filtrates		Total acid content* of stomach filtrates		pH of stomach filtrates		Peptic activity§ per kg. body weight		Pancreatic amylase activity‖ per kg. body weight
	Pyloric	Cardiac	Pyloric	Cardiac	Pyloric	Cardiac	C.G.G.†	G.M.‡	
Mean and standard error	53.2±9.8	63.4±7.4	90.7±8.0	95.5±5.1	1.7±0.2	1.6±0.2	139.0±15.1	37.5±7.3	223.0±33.7
Standard deviation	27.7	21.1	22.5	14.6	0.5	0.6	39.9	19.4	95.5
Range	20.6–91.9	31.7–99.4	58.0–125.3	76.5–122.5	0.90–2.7	0.85–2.9	98.2–189.8	10.1–57.7	91.0–357.0
No. animals	8	8	8	8	8	8	7	7	8

*Expressed as ml. of 0.1 *N* HCl per 100 ml. of filtrate.
†C.G.G.: "cardiac gland".
‡G.M.: gastric mucosae.
§One unit of peptic activity has been taken to be the hydrolytic activity of 1 mg. of the U.S.P. pepsin used in this experiment.
‖One unit of α-amylase activity has been chosen to represent the hydrolysis of 1 ml. of a 2.0% starch solution in 30 minutes at 40° C.

Nasset (7) have both shown that the secretion from the "cardiac gland" has a higher hydrogen-ion concentration than that of the stomach.

The present results indicate that the peptic activity of the "cardiac gland" is greater than that of the gastric mucosae. This finding differs from that of Smith (8) in which he showed a twofold greater activity for the stomach secretion than for the "cardiac gland" secretion.

The results of the determination of the amylolytic activity of the pancreatic gland extracts expressed as amylase units per kilogram body weight are given in Table I. The pancreatic amylolytic activity of this animal is lower than that observed for the pig (4).

The work completed to date in this laboratory suggests that there is no cellulolytic activity in the stomach contents nor in the prepared "cardiac gland" extracts of normal beavers. However, some evidence of cellulase activity was found in the caecal contents. Smith (8) observed that one of his preparations from a "cardiac gland" possessed cellulolytic activity. He was unable to confirm this finding in preparations from other animals.

It is probable that the cellulolytic activity noted by Smith (8) and by ourselves can be attributed to the commensal microflora. Work is in progress to determine if this assumption is valid.

Summary

From the present data and from those reported by Smith (8) and Nasset (7), it seems safe to conclude that gastric digestion in the beaver is similar to that in other mammals with the exception that this animal possesses a more elaborate cellular system for the secretion of pepsin. We have been unable to demonstrate the presence of a mammalian cellulase in the beaver. Some evidence was obtained that cellulolytic activity is present in the caecal contents. The pancreatic α-amylase activity is lower than that of the pig (4).

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SOMATIC METAPHASE CHROMOSOMES IN GEOGRAPHIC ISOLATES OF THE CARROT RUST FLY, CHAMAEPSILA ROSAE (F.) (DIPTERA: PSILIDAE)[1]

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Abstract

A comparative study of somatic metaphase complements of the carrot rust fly, *Chamaepsila rosae* (F.), from England, Prince Edward Island, Ontario, and British Columbia showed that the chromosome number is eight and that all chromosomes are metacentric. The means of the total complement length ranged from 50.8 to 53.5 and the lengths for chromosomal pairs I–IV averaged 36.5, 24.8, 22.3, and 16.5% of the total length respectively for the four regions. The sex chromosomes are the largest elements in the complement, the *X* chromosome being 36.5% of the total length and the *Y* 28.8%. The arm ratios for members *X*, *Y*, II, III, and IV are 1.34, 1.13, 1.57, 1.21, and 1.34 respectively. Secondary constrictions were both infrequent and irregular in location. The work emphasizes that much caution is necessary in analyzing metaphase chromosomes for taxonomic purposes.

Introduction

This is a report on the chromosomal complements of isolates of the carrot rust fly from England and three representative localities in Canada, and the taxonomic value of the data obtained is discussed. Previous work on this subject at Belleville was on certain species of Tachinidae (2) and of Sarcophagidae and Anthomyiidae (3, 4). The present work developed from biological control investigations on the carrot rust fly (Maybee, 1953). The family Psilidae is apparently unknown cytologically.

Materials and Methods

Larvae of the carrot rust fly were obtained late in 1954 from Cambridgeshire, England; Prince Edward Island; Holland Marsh, Ontario; and Lulu Island, British Columbia. The brains of third-instar larvae were stained with acetocarmine by the squash technique of Boyes and Wilkes (2) and Boyes (4). The slides were examined with a Leitz Ortholux microscope fitted with 90× oil immersion lens, 12× periplanatic eyepieces, and the Aristophot camera attachment.

The method of chromosome measurement differed from that of Boyes and Wilkes (2) in that each complement was photographed at a magnification of 1650× and printed at twice this magnification. Chromosomal features were marked by pin points along their median axes, and the distances between the points were measured directly with a rule graduated in sixty-fourths of an inch. The center of the centromere was used as the point of reference in designating the lengths of chromosome arms. Corrections were not made

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for the disparities observed in the lengths of homologous chromosomes. Boyes and Wilkes (2) commonly added or subtracted 5–10% to or from the lengths of individual chromosomes to overcome technical variation due to stretching, foreshortening, and breakages about the centromere. Such natural phenomena as differential spiralization of chromosomes and of their arms and possible chromosomal aberrations are ignored in that procedure.

The data were compiled by averaging the values for homologous chromosomes and reporting them in terms of percentage of total complement length and arm ratio. In calculating the percentage of total complement length of a heterogamete, the *Y* chromosome was not considered. Accordingly, the data represent a constructed haploid genome. Secondary constrictions were calculated from the end point of the distal arm to the end point of the proximal arm of each chromosome, and are presented as ratios of the chromosome lengths.

The data were analyzed by an analysis of variance (18, pp. 232–233) and in other ways as indicated.

Results

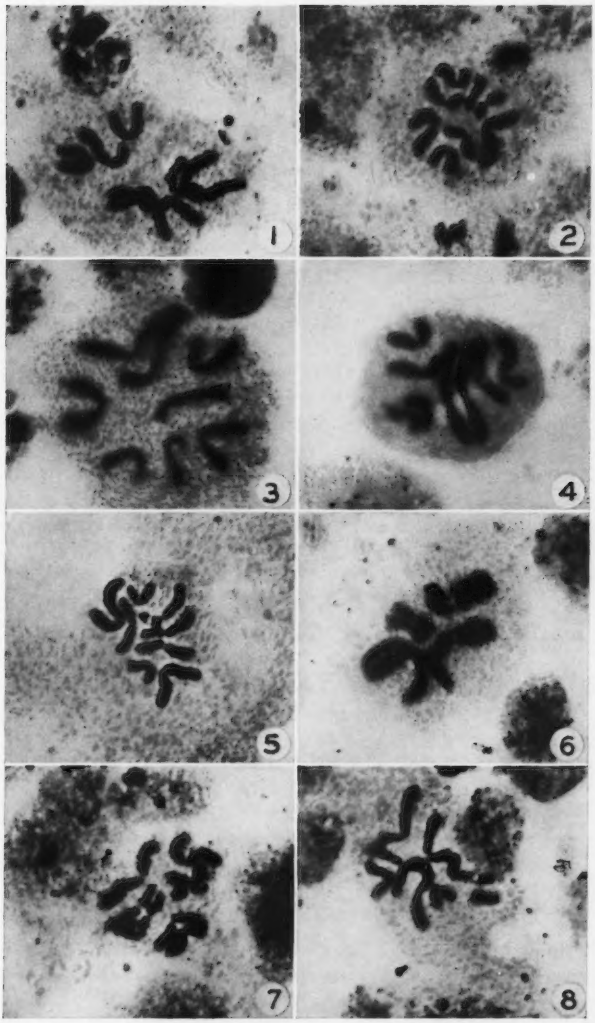
Only a fraction of a total 241 brain preparations of specimens from England (34), Prince Edward Island (57), Ontario (85), and British Columbia (65) were suitable for a study of metaphase complements. Figs. 1–8 show that the chromosome number ($2n$) is clearly eight, though in one or two cases a count of seven was obtained. The chromosomes were J-shaped and were usually associated in pairs. They generally stained deeply and uniformly, but occasionally entire complements appeared lightly stained because the complement was in early metaphase (Fig. 3). Small segments of heterochromatin appeared about the centromere and as achromatic bands (secondary constrictions).

The sex chromosomes were the largest elements in the metaphase configurations (Figs. 1–8). They were readily recognized by their strong heteromorphism in relation to recurrent differences in their lengths. For example, the length of the *Y* chromosome is 77–82% of that of the *X* chromosome (Table I). Moreover, values of the arm ratios were different (Table III). These differences were supported by the fact that the same degree of heteromorphism was present in each figure in preparations containing two to three metaphase figures. The male was assumed to be the heterogamete, on the basis of the known genetics of other Diptera.

As the extent of variation in members of homologous chromosomes has not been treated fully in the literature, the results in Table I may be of some interest. The variation (expressed as the ratio of shorter chromosome length to longer chromosome length) averaged $.93 \pm .03$ and the theoretical value of 1.00 seldom occurred.

Table II shows that the means of total complement lengths ranged from 50.8 to 53.5 μ, the differences not being statistically significant ($P > .20$).

PLATE I



FIGS. 1–8. Somatic chromosome complements at metaphase in brain cells of geographic isolates of *Chamaepsila rosae* (F.) FIGS. 1–2, England; FIGS. 3–4, Prince Edward Island; FIGS. 5–6, Ontario; FIGS. 7–8, British Columbia. Odd numbers are for male complements; even numbers, female. ×1650

The differences between mean percentages of total complement length for homologous pairs were not significantly different ($P > .20$). However, the homologues making up a chromosome set had very different values.

Table III shows that neither of the sex chromosomes had significant differences in mean values of their arm ratios ($P > .20$).

TABLE I

MEAN (±S.E.) RATIOS OF CHROMOSOME PAIRS IN ISOLATES OF *C. rosae**

Pair	England	Prince Edward Island	Ontario	British Columbia
I (Female)	.95±.02 (12)	.94±.03 (12)	.92±.02 (12)	.91±.06 (5)
I (Male)	.82±.06 (7)	.79±.03 (6)	.77±.04 (8)	.77±.01 (8)
II	.93±.02 (20)	.94±.02 (18)	.94±.02 (19)	.93±.03 (15)
III	.94±.03 (20)	.95±.02 (18)	.96±.02 (20)	.91±.04 (13)
IV	.92±.03 (20)	.94±.02 (18)	.91±.03 (20)	.88±.04 (15)

*Numbers of sets analyzed in parentheses.

TABLE II

MEAN (±S.E.) TOTAL COMPLEMENT LENGTHS (IN MICRONS) OF CHROMOSOMES AND PERCENTAGES OF TOTAL COMPLEMENT LENGTHS OF CHROMOSOME PAIRS IN ISOLATES OF *C. rosae**

Chromosome	England	Prince Edward Island	Ontario	British Columbia
No. sets	20	18	20	15
TCL	50.9±1.6	53.5±2.2	52.5±1.5	50.8±1.6
	Percentages of total complement lengths			
I	36.6± .35	36.2± .35	36.6± .43	36.5± .70
II	24.4± .21	24.8± .25	24.9± .26	25.0± .09
III	22.3± .22	22.5± .25	22.3±. 23	22.1± .44
IV	16.7± .25	16.5± .24	16.3± .08	16.5± .27

*Differences not significant ($P > .2$).

TABLE III

MEAN (±S.E.) ARM RATIOS FOR CHROMOSOMES IN ISOLATES OF *C. rosae**

Chromosome	England	Prince Edward Island	Ontario	British Columbia
XX	1.33±.03 (18)	1.34±.03 (15)	1.32±.02 (15)	1.38±.06 (8)
Y	1.08±.03 (7)	1.20±.05 (3)	1.12±.04 (7)	1.11±.04 (6)
II	1.55±.02 (19)	1.56±.04 (15)	1.61±.05 (17)	1.56±.09 (13)
III	1.22±.04 (19)	1.27±.04 (16)	1.20±.03 (15)	1.16±.04 (12)
IV	1.31±.04 (17)	1.37±.05 (15)	1.33±.04 (17)	1.36±.06 (10)

*Numbers of sets analyzed in parentheses. Differences not significant ($P > .2$).

Table IV shows that, though the values for many of the secondary constrictions were of low frequency, a secondary constriction appeared in 25% of chromosomes 7 and 8, the differences between the means not being statistically significant ($P > .20$).

TABLE IV

MEAN LOCATIONS* OF SECONDARY CONSTRICTIONS OBSERVED IN CHROMOSOMES OF *C. rosae* (TABLE I INDICATES THE NUMBER OF CHROMOSOMES EXAMINED)

(STANDARD ERRORS ARE GIVEN FOR MEANS OF CHROMOSOMES 7 AND 8)†

Chromosomes	England	Prince Edward Island	Ontario	British Columbia
X	.197 (1) .823 (2)	.210 (5) .792 (2)	— —	.253 (2) .871 (1)
3,4	— .762 (2)	— .717 (4)	.445 (6) —	.425 (1) .726 (5)
5,6	.381 (2) .698 (4)	.327 (1) —	.357 (3) .689 (1)	— .702 (1)
7,8	.356 ± .034 (7)	.375 ± .019 (9)	.371 ± .010 (13)	.369 ± .010 (7)

*Numbers of times that the locations were found in parentheses.
†Differences not significant ($P > .2$).

Discussion

The results show that chromosomal complements of larvae of the carrot rust fly from England, Prince Edward Island, Ontario, and British Columbia do not differ in number or form, but that considerable intrachromosomal variation occurs.

Brain preparations show metaphase configurations with eight metacentric chromosomes. They all show somatic pairing, a feature of chromosomal dynamics in Diptera (14). This number is characteristic of certain Drosophilidae, though 12 is the usual number found in other muscoid Diptera (2–4, 11). Because the chromosomes are metacentric, a situation in the Psilidae similar to that often noted in *Drosophila* (7, 20) may exist, namely, centric fusions may have occurred in the evolution of the family according to Robertson's "law" (17). It is possible therefore that further studies on Psilidae may reveal an ancestral species having not only higher chromosome numbers but also acrocentric chromosomes. A decrease in chromosome number may also result from unequal reciprocal translocations, as suggested from studies of the genus *Crepis* (1). However, a reduction in chromosome number is not the only possibility in the evolution of the Psilidae. For example, there may be an increase in the chromosome number such as that believed to have occurred in the evolution of the short-horned grasshoppers (Acrididae) (15).

There are no unusual heterochromatic segments in *C. rosae* such as make up sex chromosomes in some species of *Drosophila* (8, 9). The heterochromatin

appears to be confined to the region of the centromere and to secondary constrictions whose appearance in the chromosomes may be described as sporadic. This irregularity may be the result of competitive nucleination (19), and their varying locations may be attributed to differential spiralization of chromosome arms (10). However, heterochromatic segments are usually evident at metaphase only under conditions of stress (5, 6), and their location and number may depend on their association or lack of association with a nucleolus (19). In Salmonidae the secondary constrictions appear irregularly, a feature said to be characteristic of the nucleus and not of the individual (19). In the higher Diptera the location and number are variable for the most part, and, though carefully noted, were not used as taxonomic criteria (4).

Considerable differences were noted between lengths of homologous chromosomes as their mean ratio was $.93 \pm .03$ instead of the expected value of 1.00. This variation may be caused by stretching of chromosomes or of their arms and by foreshortening. As every effort was made to examine only flat chromosomes and as the preparation of slides always was done in the same manner, the technical error is considered to be comparatively small. Accordingly it may be concluded that most of the variation is an intranuclear phenomenon caused by differential spiralization (10, pp. 23–31), and/or internuclear variation where difficulties are encountered in comparing cells of the same mitotic generation (2, 19). It is because of these difficulties that data generally used in work of this type are relative length and arm ratio. These indexes did not separate the carrot rust fly into geographical races.

Boyes (4) claimed that a chromosomal separation exists at the racial and specific levels in certain anthomyiids (Diptera). These results left him "in a rather critical state of mind regarding statements often encountered in the literature of both plant and animal cytology claiming that complements of different species, races, etc. are the same or identical". However, a statistical study of the data in his Table VI (4) by a criterion (coefficient of differences) proposed for separation at subspecies levels (13) did not reveal evidence of racial or specific distinction in *Hylemya floralis* (Fall.) (= *H. crucifera* Huck.) nor could differences be found at many interspecific levels (4, Table VII).

Though Boyes' conclusions cannot be wholly accepted, the approach he used was valuable in an intensive analysis of spermatogonial chromosomes in short-horned grasshoppers (15). Powers' analyses provided a convincing demonstration of differences within individuals and between colonies, races, species, and other taxonomic entities. Elsewhere differences were found because higher taxonomic levels, such as genera in the Pyrgomorphinae (16) were being considered, or were found by sheer weight of data such as was obtained in the interspecific studies on *Crepis* (1).

This work emphasizes that much caution is necessary in the application of metaphase chromosome analyses for taxonomic purposes. Moreover, in view of the well known fact "that the similarity or dissimilarity of the

chromosomes as seen at the metaphase plate stage is not necessarily proportional to the similarity of the gene arrangements" (7), a search for inversions in salivary gland chromosomes, or chromosomal reactions on hybridization, would provide valuable confirmatory data in difficult analyses.

Acknowledgments

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PRECIPITIN TEST STUDIES ON RATE OF DIGESTION OF BLOOD MEALS IN BLACK FLIES (DIPTERA: SIMULIIDAE)[1]

A. E. R. Downe[2]

Abstract

Black fly adults were permitted to engorge on known hosts then retained for varying periods of time in a controlled laboratory incubator or under field conditions. The blood source was identifiable in all instances by precipitin tests conducted on smears 24 hours after the blood had been imbibed. Seventy-four per cent of the meals from flies held in incubators and 60% from flies held outdoors were identifiable after 32 hours, but after 40- and 48-hour intervals few meals were identifiable. The hosts used were horses and guinea pigs. It is tentatively concluded from a comparison of data and from field- and incubator-held flies that temperature influences the rate of digestion.

Introduction

Recent studies on identification of blood meals of some species of black flies by the precipitin test (1) required further investigations on the rate of digestion of blood in these insects. It is important, in interpretation of blood-meal studies, to know the length of time taken to so alter the serum proteins of the blood meal in the digestive tract of an insect that they can no longer be identified by serological methods. Also, knowledge of the rate of digestion has value in studies of the general feeding habits of bloodsucking insects and their roles as vectors of diseases.

The precipitin test has been used to determine rates of digestion of blood in mosquitoes by West and Eligh (5) and O'Gower (3) and in other haematophagous arthropods by Weitz and Buxton (4). No records of similar studies on black flies were found in the literature.

This is a report on precipitin test studies on the rate of digestion of blood meals in black flies collected at Rapides des Joachims, Quebec, and in the Ottawa Valley, Ontario, during the summers of 1954 to 1956. The term *rate of digestion* was defined by O'Gower (3) as the period of time for blood meals of various insect species to reach such a stage of digestion that the serum proteins no longer precipitate their specific antibodies.

Materials and Methods

Blood-engorged black flies that had completed their feeding were collected from horses and guinea pigs with an aspirator. These flies were placed in cloth-covered cages (12 in. × 14 in. × 18 in.) with xylonite fronts and given 10% sucrose solution. Some of the cages were left in a field, where they were partially sheltered by low bushes. Others were taken to the laboratory

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and placed in an incubator at 67°–70° F. and 75–80% relative humidity. Temperature and humidity records were taken in the field areas where caged specimens were kept (Table II).

At intervals of 8 hours, samples of the specimens were removed from the cages, identified to species, and smeared on strips of filter paper. Three to four weeks later, the filter-paper smears were extracted with physiological saline (pH 7) and subjected to precipitin tests ("ring" method) with appropriate antisera by the method of Eligh (2).

Results and Discussion

The blood meals of specimens held in the laboratory or under field conditions could be identified consistently after 24 hours (Tables I and II). Many of the specimens (about 74%) kept in the laboratory incubator at relatively constant temperature and humidity gave positive reactions after 32 hours, but fewer positive reactions were obtained from specimens killed 40 to 48 hours after they had fed. No positive reactions were obtained after 48 hours.

There were no striking differences in the rates of digestion of the four species of black flies listed in Tables I and II, although small differences may exist. O'Gower (3) reported differences in the rate of digestion of blood in some species of mosquitoes.

One difficulty encountered was the failure of the flies to survive for adequate periods, particularly in the laboratory. Under the laboratory and field conditions already described, about 60% of each collection survived for 65 hours. Blood-engorged specimens kept in an insect rearing room at 80° F. and 70–80% relative humidity or in a refrigerated cabinet at 40° F. nearly all died after 24–40 hours, so that a thorough study of effect of temperature on rate of digestion could not be made. Table II indicates, however, that the rate of digestion is accelerated when temperatures are comparatively

TABLE I

RESULTS OF PRECIPITIN TESTS ON SMEARS MADE FROM BLACK FLIES RETAINED IN A LABORATORY INCUBATOR* FOR VARYING PERIODS AFTER THEY HAD FED

Species	Source of blood meal	Number positive out of number tested after: 8 hr.	16 hr.	24 hr.	32 hr.	40 hr.	48 hr.
Simulium venustum Say	Horse	19/19	13/13	19/20	16/20	9/20	0/5
	Guinea pig	5/5	5/5	8/8	5/6	0/2	0/2
S. vittatum Zett.	Horse	15/15	20/20	20/20	18/25	6/14	1/15
	Guinea pig			5/5	3/3	0/5	
Prosimulium hirtipes (Fries)	Horse	12/12	14/15	20/20	15/20	1/3	
S. parnassum Mall.	Horse	5/5	10/10	6/6	2/3	2/5	0/5
	Guinea pig			5/5	4/5	1/2	0/3

*Incubator operated at 67–70° F. and 75–80% relative humidity.

TABLE II

POSITIVE REACTIONS IN PRECIPITIN TESTS ON BLOOD MEALS OF BLACK FLIES AFTER VARIOUS PERIODS OF DIGESTION UNDER FIELD CONDITIONS

Species	Temperature, °F.			Humidity range, %	Source of blood meal	Number positive out of number tested after:					
	Mean	Max.	Min.			8 hr.	16 hr.	24 hr.	32 hr.	40 hr.	48 hr.
Similium venustum Say	66°	72°	54°	63–84	Horse	16/16	16/16	17/17	6/12	2/6	
	66°	72°	54°	63–84	Guinea pig	5/5	5/5	3/3	2/4	0/8	
	51°	59°	47°	51–88	Guinea pig			4/4	3/3		2/4
S. vittatum Zett.	83°	97°	74°	61–89	Horse	10/10	5/5	8/10	2/10	0/8	0/4
	83°	97°	74°	61–89	Guinea pig			7/7	1/4	0/5	
	66°	72°	54°	63–84	Horse	3/3	4/4	4/4	4/6	1/3	0/5
	66°	72°	54°	63–84	Guinea pig	5/5	5/5	7/8	1/4	1/4	
	51°	59°	47°	51–88	Guinea pig			5/5	8/10	3/4	1/2
Prosimulium hirtipes (Fries)	74°	81°	53°	68–90	Horse	5/5	5/5	8/8	6/14	0/4	0/2
	66°	72°	54°	63–84	Horse		8/8	10/10	7/12	3/12	0/3
	51°	59°	47°	51–88	Horse			10/10	8/10	3/9	2/7
S. parnassum Mall.	83°	97°	74°	61–89	Horse		6/6	9/9	2/6	0/11	
	51°	59°	47°	51–88	Horse			10/10	5/6	2/3	

high and noticeably retarded at low mean temperatures. This is also reported to occur in mosquitoes (3, 5). Humidity was not sufficiently varied to allow an adequate appraisal of its effect on rate of digestion, but the results from the tests on specimens kept at constant relative humidity in the laboratory are similar to those from tests of specimens held in the field under varied humidity conditions in the same temperature range.

Although serum proteins of blood meals were not usually detected by their corresponding antibodies 32 hours after ingestion, a residuum of blood was found in the ventriculus of dissected specimens of *S. venustum* kept in the laboratory incubator up to 65 hours after feeding. Eight of these specimens were subjected to the sensitive Meyer reduced phenolphthalein test for blood 50 hours after they had fed on horse blood, and five gave positive results. No positive results were obtained when 11 specimens of the same species were tested 60 hours after they had fed, indicating the virtual completion of the digestion process.

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DECAPOD CRUSTACEA OF THE CALANUS EXPEDITIONS IN UNGAVA BAY, 1947 TO 1950[1]

"CALANUS" SERIES NO. 11

H. J. SQUIRES

Abstract

The decapod fauna of Ungava Bay (17 species in 3000 specimens collected) is shown to be similar to that of the shallow water areas of west Greenland. Four species are reported for the first time from Ungava Bay: *Sergestes arcticus* and *Pasiphaea tarda*, ordinarily from deeper and warmer water, and *Eualus macilentus* and *Sabinea septemcarinata*. Species found in or originating in the Pacific were taken in greater numbers. Systematics of each species is treated under occurrence in Ungava Bay, world distribution, and taxonomy. Lengths of most species of shrimp showed that a greater size was reached in females. Maturities with respect to size when first mature, egg size, and times of hatching and spawning are discussed. Males were found to be mature at a size smaller than first-mature females. The high percentage of stations at which decapods, including larvae, were taken, and their occurrence in the stomachs of many seals and fish attest their prevalence and their importance in the area.

Introduction

Previous to the work of the *Calanus* Expeditions, 1947 to 1950, in Ungava Bay, few collections of decapods had been made in this area. A synopsis of the species taken and the names of their collectors is as follows:

Dr. Robert Bell, in 1884, took the following decapods in dredgings at Port Burwell (Smith, 1885 (14)):

4	*Eupagurus krøyeri* Stimpson	
1 F	*Ceraphilus boreas* (Phipps)	= *Sclerocrangon boreas*
2 F	*Hippolyte fabricii* (Krøyer)	= *Eualus fabricii*
1 M, 11 F	*H. phippsii* (Krøyer)	= *Spirontocaris phippsi*
8 M, 7 F	*H. groenlandicus* (J. C. Fabricius)	= *Lebbeus groenlandicus*
5 M, 11 F	*H. polaris* (Sabine)	= *L. polaris*
1 F	*Pandalus montagui* Leach	

A collection by Lucien M. Turner in 1882 to 1885, in Ungava Bay, comprised the following species (Rathbun, 1913 (12)):

Sclerocrangon boreas (Phipps)	
Spirontocaris polaris (Sabine)	= *Lebbeus polaris*
S. fabricii (Krøyer)	= *Eualus fabricii*

In 1884, A. P. Low made dredgings "on the south side of Hudson Strait, between King George Sound and the bottom of Ungava Bay" (Whiteaves, 1901 (20)). Whiteaves, however, reported only *Pandalus montagui* from Hudson Strait as being from Low's collection. No further reference to Low's collection has been seen by the present author.

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The *Diana* Expedition, 1897, collected in Ungava Bay the following species (Rathbun, 1919 (12)):

3 *Eupagurus krøyeri* Stimpson
1 *Hyas coarctatus* Leach

The *Neptune* Expedition, 1903 to 1904, took the following species at Port Burwell (Rathbun, 1919 (12)):

12	*Spirontocaris groenlandicus* (Fabricius)	= *Lebbeus groenlandicus*
1	*S. spina* (Sowerby)	= *S. spinus*
1	*S. lilljeborgi* (Danielssen)	
23	*S. phippsi* (Krøyer)	
6	*S. polaris* (Sabine)	= *Lebbeus polaris*
17	*S. fabricii* (Krøyer)	= *Eualus fabricii*
20	*S. gaimardi* (Milne-Edwards) (varying toward *S. g. belcheri*)	= *E. gaimardi* and *E. g. belcheri*
1	*Sclerocrangon boreas* (Phipps)	
	Nectocrangon lar (Krøyer)	= *Argis dentata* Rathbun

The *Calanus* Expeditions, 1947 to 1950, in Ungava Bay, took 17 species in all, which comprise, in addition to the species collected previously in this area, the following four species:

Sergestes arcticus Krøyer
Pasiphaea tarda Krøyer
Eualus macilentus (Krøyer)
Sabinea septemcarinata (Sabine)

In addition, the *Calanus* Expeditions collected larvae of species mentioned in the families Pandalidae, Hippolytidae (most species), and Crangonidae (except *Sclerocrangon*), as well as larvae of *Eupagurus krøyeri* and *Hyas coarctatus*.

A more recent collection (August 9 to 28, 1954) of decapods from Brunnich's murre food to young on Akpatok Island, Ungava Bay, yielded the following species (Tuck and Squires, 1955 (19)):

7 *Pandalus montagui* Leach
14 *Lebbeus polaris* (Sabine)
1 *L. groenlandicus* (Fabricius)
1 *Spirontocaris spinus* (Sowerby)
55 *Argis dentata* Rathbun

Areas adjacent to Ungava Bay have been collected in very extensively to the east (Davis Strait, Baffin Bay, and particularly west Greenland) but less extensively to the west (Hudson Bay). Some species collected in Hudson Bay and Strait to which Whiteaves (1901 (20)) refers are:

Pandalus montagui
Spirontocaris phippsi
Sclerocrangon boreas

Hyas coarctatus was mentioned by Rathbun (1925 (12)) as collected by Preble in 1900.

The *Neptune* Expedition also collected in Hudson Bay the following species (Rathbun, 1919 (12)):

Lebbeus groenlandicus
L. polaris
Eualus fabricii
E. gaimardi

West Greenland and adjacent areas have been explored very extensively, and decapod species were collected by the *Alert* (British Arctic Expedition, 1875 to 1876), *Ingolf* Expedition (1895 to 1896), the Godthaab Expedition (1928), Treaarexpeditionen til Christian d. X's Land (1931 to 1934), 6th and 7th Thule Expeditions (1933), and *Dana* Expedition (1933) and were taken in many other smaller collections, all of which are reviewed by Hansen (1908 (8)), Stephensen (1935 (15)), and Heegard (1941 (9)). Species collected from west Greenland, Davis Strait, and Baffin Bay may be separated according to whether they were taken in shallow or deep water, as follows:

Shallow water species	Depths, meters	Shallow water species	Depths, meters
Pandalus montagui	64	*E. macilentus*	16–100
Lebbeus microceros	—	*Sabinea septemcarinata*	10–100
L. groenlandicus	0– 50	*Argis dentata*	0–100
L. polaris	4–350	*Sclerocrangon boreas*	0–150
Spirontocaris phippsi	4– 70	*Eupagurus pubescens*	0–200
S. lilljeborgi	12– 16	*Hyas araneus*	60–160
S. spinus	8– 50	*H. coarctatus*	0–150
Eualus fabricii	4– 50	*Chionoecetes opilio*	160
E. gaimardi (incl. *belcheri*)	12–100		

Deep water species		Depths, meters	Deep water species	Depths, meters
Sergestes arcticus	(pelagic)	500–1600	*B. payeri*	450– 820
Gennadas elegans	(")	500–2800	*Pontophilus norvegicus*	500– 800
Hymenodora glacialis	(")	250–2000	*Sabinea sarsi*	150– 200
Parapasiphaë sulcatifrons	(")	1000–2700	*S. hystrix*	750–1100
Pasiphaea tarda	(")	800–1200	*Sclerocrangon ferox*	450– 880
Ephyrina benedicti	(")	300–3500	*Polycheles nanus*	1600
Acanthephyra multispina	(")	300–3000	*Munidopsis antoni*	2400
Pandalus propinquus		600–1400	*M. curvirostra*	650– 750
P. borealis		150– 700	*Munida tenuimana*	500–1200
Bythocaris gracilis		700	*Lithodes maja*	140– 550
B. leucopis		140– 500	*Neolithodes grimaldi*	1000

It may be seen that almost all the shallow water species from west Greenland were also taken in Ungava Bay (except *L. microceros*, *Eupagurus pubescens* (Hansen, 1908 (8), believes this to be synonymous with *E. krøyeri*), *Hyas araneus*, and *Chionoecetes opilio*). However, the pelagic deep-water species *Pasiphaea tarda* and *Sergestes arcticus* were taken in shallower waters than reported heretofore, near the entrance to Ungava Bay by the *Calanus* Expeditions, 1947 to 1950.

Key to Species

The following artificial key to species collected, and allied species which occur in areas adjacent to Ungava Bay, has been compiled from several sources (chiefly, Sund, 1912 (16); Schmitt, 1921 (13); Rathbun, 1929 (12);

Holthuis, 1947 (10), 1955 (10)). The numbers of epipods on periopods, or the presence of exopods on the third maxilliped in some species have been included, because separation of species is often not otherwise possible in damaged specimens from stomach contents of fish and seals.

1*a*. Body laterally compressed. Pleopods used for swimming.....Suborder NATANTIA—2

2*a*. First three pairs of periopods chelate; pleura of second segment of abdomen not overlapping those of the first or third segments. Gills dendrobranchiate..... Tribe PENAEIDEA—3

3*a*. Last two pairs of legs as well developed as the first three pairs..Family Penaeidae

3*b*. Last one or two pairs of legs not as large as the first three pairs, rudimentary or wanting.....Family Sergestidae

a. Rostrum very short. One antennular flagellum very long, the other very short. First joint of antennular peduncle much longer than the third..*Sergestes arcticus*

2*b*. First two pairs of periopods chelate; pleura of second segment of abdomen overlapping those of the first and third segments. Gills phyllobranchiate...Tribe CARIDEA—4

4*a*. Exopods on all periopods. Cutting edge of chela pectinate..... .....Family Pasiphaeidae—5

5*a*. Basis of second periopod with 7 to 12 spines.....*Pasiphaea multidentata*

5*b*. Basis of second periopod with one to four spines.....*Pasiphaea tarda*

4*b*. No exopods on periopods..... 6

6*a*. First periopods chelate..... 7

7*a*. Rostrum laterally compressed, long, armed above with spines, moveable for the most part, and armed with fixed teeth below. First two pairs of periopods greatly unequal, carpus of second pair annulated. Mandible with palp.....Family Pandalidae—8

8*a*. Third abdominal segment somewhat carinated dorsally and armed with a short spine or lobe. Carpus of right second periopod with about 25 annulations.....*Pandalus borealis*

8*b*. Third abdominal segment without carina or lobe or spine..... 9

9*a*. Carpus of right second periopod with about five annulations.... .....*Pandalus propinquus*

9*b*. Carpus of right second periopod with about 20 annulations..... .....*Pandalus montagui*

7*b*. Rostrum toothed; no moveable spines, usually well developed, sometimes reduced. Right and left periopods equal, first pair stouter and usually shorter than the second; carpus of second pair of periopods with seven annulations.....Family Hippolytidae—10

10*a*. Supraorbital spines present on carapace.....11

11*a*. Carapace with two supraorbital spines at each side. Third maxilliped with an exopod.....*Spirontocaris*—12

12*a*. Rostrum with equal teeth above and below, and extending on to carapace to anterior third....*Spirontocaris phippsi*

12*b*. Rostrum with unequal teeth.....13

13*a*. Teeth of rostrum continued on carapace reaching almost to posterior margin. Rostrum short, ending in an arcuate gap between two spinous tips..... *S. spinus*

13*b*. Teeth dorsally on carapace not nearly reaching posterior margin; rostrum ending in one long point .....*S. lilljeborgi*

11*b*. Carapace with one supraorbital spine on each side. Third maxilliped without an exopod.....*Lebbeus*—14

14*a*. Pleura of abdominal segments armed laterally with spines. Epipod on first three periopods.....*Lebbeus groenlandicus*

14*b*. Pleura of abdomen rounded, unarmed.....15

15*a*. Rostrum as long as antennular peduncle. Epipod on first two periopods.....*L. polaris*

15*b*. Rostrum not exceeding first segment of antennular peduncle. Epipod on first three periopods..... .....*L. microceros*

10*b*. Carapace with no supraorbital spines. Third maxilliped with an exopod.....*Eualus*—16

16*a*. Rostrum about as long as rest of carapace.....17

17*a*. Terminal half of rostrum without spines above. Epipod on first periopod only.....*Eualus fabricii*

17*b*. Terminal half of rostrum with spines above. Epipods on first two periopods.....18

18*a*. Tubercle dorsally on third abdominal segment, mostly with a strong hook.....*E. gaimardi belcheri*

18*b*. No tubercle dorsally on the third abdominal segment .....*E. gaimardi*

16*b*. Rostrum shorter than rest of carapace.....*E. macilentus*

6*b*. First periopods subchelate. Rostrum when present generally small, usually dorsally flattened.....Family Crangonidae—19

19*a*. Second pair of periopods chelate.....20

20*a*. Dactyls of fourth and fifth periopods not dilated, not natatorial. Carapace with strong sculpture.....*Sclerocrangon*—21

21*a*. Rostrum short, horizontal above, an axe-shaped expansion forming its keel.....*S. boreas*

21*b*. Rostrum longer with tip ascending, expansion below also pointed anteriorly, obliquely downward.....*S. ferox*

20*b*. Dactyls of fourth and fifth periopods dilated, natatorial..... .....*Argis*—22

22*a*. Carinae on sixth abdominal segment each forming a tooth directed posteriorly.....*A. dentata*

22*b*. Carinae on sixth abdominal segment rounded posteriorly, each not forming a tooth.....*A. lar*

19*b*. Second pair of periopods not chelate, smaller than the first, rudimentary .....*Sabinea*—23

23*a*. Rostrum obtuse.....*S. septemcarinata*

23*b*. Rostrum acute.....*S. sarsi*

1*b*. Body generally depressed. Abdominal appendages reduced, sometimes absent, not used for swimming.....Suborder REPTANTIA—24

24*a*. Carapace not fused with epistome. Abdomen anomurous, showing some traces of function other than that of reproduction; asymmetrical, biramous limbs on sixth segment.....Tribe ANOMURA

Uropods present, modified for holding in hollow objects; abdomen soft, showing no trace of segmentation. Hermit crabs.....Family Eupaguridae—25

25*a*. Left hand with outer margin inflexed, a well-defined ridge in middle with one principal row of spines; larger face concave.....*Eupagurus krøyeri*

25*b*. Left hand with outer margin arcuate, not strongly ridged, a double row of spines on crest at middle; larger face convex.....*Eupagurus pubescens*

24*b*. Carapace fused with epistome, at least at sides. Abdomen brachyurous, small, straight, symmetrical, bent under thorax, and without biramous limbs on sixth segment.....Tribe BRACHYURA

Fore part of body narrow, forming a distinct rostrum.....Family Majidae—26.

26*a*. Carapace about as long as broad. Basal article of antenna long and narrow .....*Chionoecetes opilio*

26*b*. Carapace much longer than broad; rostrum elongate.....*Hyas*—27

27*a*. Carapace subtriangular; basal article of antenna triangular, pointed anteriorly, smooth.....*Hyas araneus*

27*b*. Carapace lyrate; basal article of antenna almost rhomboidal, narrowing anteriorly, knobbed.....*Hyas coarctatus*—28

28*a*. Carapace to rostrum length, 4.5 to 6.4 : 1.....Forma *typica*

28*b*. Carapace to rostrum length, 7.1 to 9.3 : 1.....Forma *alutaceus*

Methods

Adults examined for this paper were taken by dredge (steel mesh in 1947, 1948, and 1949; and with rope bag in 1950, which was used in an open meshless frame and enclosed in the wire-meshed dredge in most sets), beam-trawl (a few sets only), stramin net which touched bottom, fine-meshed nets Nos. 5, 00, and 6 which touched bottom inadvertently in plankton towing, No. 00 towing in current at 40 meters (bottom depth 185 meters), and shrimp net on bottom. A considerable number of specimens were taken also from stomach contents of cod (*Gadus callarias* Linné), sculpin (? *Myoxocephalus groenlandicus* Cuvier & Valenciennes), bearded seal (*Erignathus barbatus* (Erxleben)), ringed seal (*Phoca hispida* Schreber), harbor seal (*Phoca vitulina* Linné), and harp seal (*Phoca groenlandica* Erxleben). A few specimens of *Hyas* sp. were taken also by hand on the shore.

Specimens, eggs, etc., were measured as preserved in about 7% formalin. Carapace lengths were measured from the posterior margin of the carapace to the posterior margin of the orbit (Chace, 1940 (1)), and total lengths from the tip of the rostrum to the tip of the telson, all with vernier calipers: the calipers were adjusted to the length of carapace under low power of a dissecting microscope in very small specimens (carapace lengths 3 to 4 mm.). Only animals in good condition were measured; many from stomach contents could not be measured. The greater diameter of an egg was measured directly at a magnification of 10×, estimated to the nearest tenth of a millimeter on a Turtox millimeter grid which had each printed line center scored with a sharp-pointed scalpel. Nonovigerous and ovigerous females were slit open and a few eggs removed from the ovary for measuring; largest eggs from the ovary and pleopods were used for the egg measurements recorded for each specimen.

Sex was determined from examination for appendix masculina in shrimp or prawns and spider crabs, appendages and position of sexual ducts in hermit crabs, and petasma in *Sergestes* sp. Sizes of appendices masculinae and eggs, or presence of eggs on pleopods, were used for a gross determination of maturity.

The number of each station referred to in the systematic account is given in the station lists of the *Calanus* Expeditions, 1947 to 1950 (Dunbar and Grainger, 1952 (4); Grainger, 1954 (7)). Numbers of specimens and of each sex are given following the station number—the number of specimens in parentheses when not the same as the number sexed. The definition of "arctic", "subarctic", and "boreal" follows that given by Dunbar (1953 (3)).

Synopsis of the Species

The following species were taken in Ungava Bay. World distribution and some variations in taxonomy of the specimens examined is indicated.

Family SERGESTIDAE

1. *Sergestes arcticus* Krøyer, 1885. Sund, Oscar, 1920; p. 8, Fig. 5; Hansen, H. J., 1908, p. 82

In all, 267 specimens were taken. Of these, 88 were taken by shrimp net and the rest from stomach contents, as follows: cod, 174; harp seal, 4; and ringed seal, 1. Seventy-one males and 80 females examined and measured were all adult and ranged from 9 to 16 mm. in carapace length. Depths where cod were taken were 15 to 37 meters, and the shrimp net fished at 27 to 37 meters. No larvae of *Sergestes* were taken in plankton net tows.

Occurrence at stations:
1947—44: 1M, 1F; 45: (24) 3M, 17F
1948—Tunnusaksuk Fjord: (1); 74, 77, and 78: (2) 1F; between Bush and Killinek Islands: (4)
1949—105: 13M, 13F
1950—Resolution Island, AS28: (110) 47M, 38F; AS28: (88) 17M, 23F; AS28: (10) 3M, 4F

Bathypelagic in subarctic and boreal waters in the Atlantic only. East and west Greenland to Nova Scotia and mid-Atlantic; coast of Norway, 65° 20′ N., and south to the western part of the Mediterranean; a few were taken to the south of Australia by the *Challenger* (Heegard, 1941 (9, p. 61, map Fig. 27)). The *Calanus* Expeditions extend the range of this species westward to Hudson Strait (Resolution Island) and to Port Burwell and Tunnusaksuk Fjord in Ungava Bay, in shallower water than reported heretofore.

Family PASIPHAEIDAE

2. *Pasiphaea tarda* Krøyer, 1854. Sund, Oscar, 1912

The only way of collecting this species by the *Calanus* Expeditions was from stomach contents of Atlantic cod (*Gadus callarias* L.), and 46 specimens in all were collected. Six males and 17 females examined were adult, ranging in carapace lengths from 18 to 44 mm. No larvae of this species were taken. Cod were taken in depths of 15 to 79 meters.

Occurrence at stations:
1947—44: (1)
1949—105: (36) 3M, 14F
1950—AS28: 3M, 3F

A north Atlantic bathypelagic species in the subarctic and boreal areas, never in the Arctic; northern Europe to Ireland; Iceland; southeast and west coasts of Greenland and Davis Strait (J. G. De Man, 1920 (2)). Heegard (1941 (9)) gives as a record of distribution the east coast of North America at Massachusetts, but this must be from Smith (1879 (14)), who considered *P. multidentata* to be synonymous with *P. tarda*. *P. multidentata*, however, is described as a distinct species by most authors; it has been taken occasionally off Newfoundland as has *P. tarda* also, from deep water (H. J. Squires, unpublished). The *Calanus* Expeditions extend the distribution of *P. tarda* to the northeast coast of America at Resolution Island and Port Burwell, and in shallower water than reported heretofore.

FAMILY PANDALIDAE

3. *Pandalus montagui* Leach, 1814. Rathbun, M. J., 1929, p. 8, Fig. 5

Eighty-one specimens were taken throughout the area: 3 by stramin net which touched bottom, 11 by dredge and beam-trawl; and 37 from cod, 29 from ringed seal, and 1 from bearded seal stomach contents. In good condition when examined were 23 males, 10 to 18 mm., and 17 females, 20 to 25 mm. in carapace length. Depths, 15 to 275 meters. Temperatures, −1.39 to −1.00° C. Salinities, 31.87 to 33.53‰.

Occurrence at stations:
1947—18: 1M, 1F; 45: 4M; 45: (17) 4M, 10F
1948—Burwell Hr.: (4); 103: 3M, 2F; 105: (17) 7M, 7F; 107: 2M
1950—Cape Hopes Advance: (26) 1M; 208: 1M; 224: (1); 224: (2) 1F

Subarctic and boreal (Stephensen, 1935 (15)); part of the endemic archibenthal fauna of the North Atlantic (Ekman, 1953 (5)). White Sea, Murman Sea; from the extreme north of Norway to the English Channel: the whole of the North Sea, Skagerrak, Kattegat, and most western part of the Baltic, Rockall; around the coasts of Iceland; Baffin Bay; east coast of North America as far south as latitude 41° 25′ N. Depths 15 to 290 meters. (De Man, 1920 (2).)

Spines on the rostrum $\frac{6 - 17}{4 - 6}$, two to seven of which are on the carapace in these specimens; given for other areas $\frac{12 - 16}{6 - 9}$, three to four of which are on the carapace (Rathbun, 1929 (12)). Four to six pairs of spines laterally on the telson, sometimes not paired.

FAMILY HIPPOLYTIDAE

4. *Spirontocaris lilljeborgi* (Danielssen, 1859). Rathbun, 1929, p. 14, Fig. 13; Holthuis, 1947, p. 8

Only two specimens of this species were taken—in the stomach contents of bearded seals. One male was adult at 7 mm. carapace length.

Occurrence at stations:
1948—15 miles ENE. of the Gyrfalcon Islands: 1M
1949—25 miles off Payne Bay: (1)

Subarctic and boreal; Europe, from the Murman Sea south to the Kattegat; in Davis Strait and North America from Nova Scotia to 37° N. (Heegard, 1941 (9)); north coast of Alaska (Rathbun, 1904 (12)). The collections of the *Calanus* Expeditions extend the range of this species to Ungava Bay.

Hansen (1908 (8, p. 60)) states that this species is boreal and not arctic, but refers to Rathbun's record on the north coast of Alaska at 19 fathoms (Rathbun, 1904 (12, p. 68)). Stephensen (1935 (15, p. 83)) calls this species boreal and accidental in low-arctic waters. Heegard (1941 (9)) also ignores the Alaska record, calling it boreal. Ekman (1953 (5, p. 153)) gives this species as an example of boreal submergence.

5. *Spirontocaris phippsi* (Krøyer, 1841). Holthuis, 1947, p. 8; Rathbun, 1929, p. 13, Fig. 12

S. turgida. Hansen, 1908; Heegard, 1941; Ekman, 1953

In all, 137 specimens were taken: 21 by stramin net which touched bottom; 24 by dredge; and 18 in cod, 32 in ringed seal, 36 in bearded seal, and 6 in harbor seal stomach contents. Depths, where taken by dredge, were 14 to 110 meters. Temperatures, −1.39 to 2.07° C. Salinities, 29.40 to 33.42°/₀₀.

Occurrence at stations:

1947—13: (11) 1M, 6F; 18: (10) 3M, 6F; 33: 2M, 2F; 45: 3F; 45: (6) 1M, 4F; 48: 1F

1948—Koksoak River mouth: (34) 1M, 32F; 15 miles ENE. Gyrfalcon Islands: (2) 1F; 58: 2F; 59: 4F; off Whale River: (28) 11F

1949—105: (9) 1M, 7F; Button Islands: (6) 1F; 126: 2F

1950—Cape Hopes Advance: (1); 202: (3) 2F; 208: 1M; 210: 5F; 222: 3F; 224: 2F

Circumpolar, in subarctic waters; southward to northern Norway; Cape Cod (east coast of North America) and Plover Bay (Siberian east coast). Depths 11 to 225 meters. (Holthuis, 1947 (10).)

Spines on rostrum $\frac{4 - 18}{3 - 8}$, zero to six of which are on the carapace (somewhat less in males than females) in these specimens; $\frac{7 - 12}{4 - 7}$, four to five of which are on the carapace, has been given for other areas (Rathbun, 1929 (12)). Two to seven pairs of spines laterally on the telson, often not paired completely.

6. *Spirontocaris spinus* (Sowerby, 1805). Holthuis, 1947, p. 8

S. spina. Rathbun, 1929, p. 14, Fig. 14

In all, 185 specimens were taken: 77 by dredge and beam-trawl; 34 by stramin net which touched bottom; and, in stomach contents, 47 in cod and 21 in bearded seal, 3 in ringed seal, and 3 in harbor seals. Depths, where taken by dredge, were 9 to 275 meters. Temperatures, −1.39 to 3.10° C. Salinities, 29.40 to 33.42°/₀₀.

Occurrence at stations:

1947—3: 1F; 11: 1F; 13: 3M, 5F; 18: (8) 2M, 4F; 33: 4M, 3F; 44: (2); 45: (9) 4M, 4F; 48: 1M, 1F

1948—Off Koksoak River: (1); 15 miles ENE. Gyrfalcon Islands: (3) 2F; 59: 2F

1949—103: 3M, 16F; 106: 1F; 107: 2M, 2F; Port Burwell: (1); 105: (1); 105: (22) 4M, 9F; Button Islands: (3); off Payne Bay: (1); 126: (1); 128: 11M, 7F

1950—Cape Hopes Advance: (5); 201C: 1F; 202: (12) 2M; 203: 2F; 208: 2M, 5F; 210: 2M, 3F; 222: 9M, 15F; 224: 2F; 226: 1M, 1F; 224: (10) 2M, 5F

Circumpolar, subarctic, and boreal; southward to the northern North Sea; Iceland; Greenland; North America, Massachusetts Bay to the Behring Sea, Alaska Peninsula; Siberian east coast. Depths 16 to 400 meters (Heegard, 1941 (9); Holthuis, 1947 (10)). This species is also denoted as a constituent of the archibenthal fauna (Ekman, 1953 (5)).

Major spines on rostrum $\frac{8 - 28}{1 - 5}$, 4 to 21 of which are on the carapace in these specimens; given for other areas $\frac{9 - 33}{2 - 5}$, with an average of 18 to 19 above (Rathbun, 1929 (12)). The spines counted above on the rostrum and carapace are major ones only; there are very many secondary, serrulate spines in addition. Four to 10 spines laterally on the telson, often not always paired.

7. *Lebbeus groenlandicus* (J. C. Fabricius, 1775). Holthuis, 1947, p. 9
Spirontocaris groenlandica. Rathbun, 1929, p. 11, Fig. 8

In all, 413 specimens were taken: 159 by dredge and beam-trawl; 7 by stramin net which touched bottom; 2 by shrimp net on the bottom; and 40 in cod, 176 in bearded seal, 14 in ringed seal, and 14 in harbor seal stomach contents. Depths, where dredged, etc., 18 to 275 meters. Temperatures, −1.39 to 2.16° C. Salinities, 28.99 to 33.53‰.

Occurrence at stations:

1947—3: 2M, 2F; 11: (7) 2M, 3F; 13: 1M, 1F; 18: (1); 20 and 21: 3F; 28: 6M, 8F; 30: 20M, 13F; 33: 2M, 6F; 45: (11) 1M, 7F; 48: 3M, 1F

1948—30 miles toward Burwell from George River: (2); Koksoak River mouth: (12) 3F; 53: 1M; 15 miles ENE. Gyrfalcon Islands: (39) 8M, 4F; 58: 6M, 1F; 59: 4M, 5F; off Whale River: (45) 4F

1949—103: 10M, 16F; 106: 3F; 105: (14) 3M, 7F; Button Islands: (15) 6F; off Payne Bay: (5); 126: 3M, 3F; 103: 1M, 3F

1950—Cape Hopes Advance: (3); 202: (69) 2M, 10F; 205: (2); 206: 2M; 208: 1M, 2F; 210: 3M, 8F; 212: (4); 216: 1F; 222: 5M, 3F; 224: (13) 3M, 3F; 226: 4M, 6F; 236: (9) 1F; AS28: (3) 1M, 1F

Arctic, subarctic, and boreal; Pacific (Heegard, 1941 (9)). East and west Greenland, southward to Massachusetts Bay; arctic Canada; Behring Sea to Puget Sound; Sea of Okhotsk. Depths 2 to 210 meters (Holthuis, 1947 (10)).

Spines on rostrum $\frac{1 - 4}{1 - 4}$, plus four spines invariably on the carapace in these specimens; $\frac{2 - 3}{2 - 3}$, plus four spines invariably on the carapace, has been given for other areas (Rathbun, 1929 (12)). Six to seven spines laterally on the telson (Rathbun, 1929 (12)), 4 to 10 laterally on telson in the specimens examined.

8. *Lebbeus polaris* (Sabine, 1821). Holthuis, 1947, p. 9
Spirontocaris polaris. Rathbun, 1929, p. 12, Fig. 9

As in west Greenland (Stephensen, 1935 (15)) this was the most abundant species taken in Ungava Bay by the *Calanus* Expeditions.

In all, 473 specimens were taken: 211 by dredge and beam-trawl; 23 by stramin net which touched bottom; 2 by plankton net No. 00 at 40 meters (not on bottom); 123 by plankton net No. 5 which touched bottom; 19 by shrimp net; and 69 in cod, 13 in ringed seal, 8 in bearded seal, and 5 in harbor seal stomachs. Depths, where taken by dredge, etc., 5 to 275 meters. Temperatures observed, −1.39 to 3.40° C. Salinities, 29.40 to 33.53‰.

Occurrence at stations:

1947—3: 4F; 7: 3M, 4F; 11: 3M; 18: (5) 4F; 20 and 21: 2M, 2F; 28: 2F; 33: 1M; 45: (37) 12M, 22F

1948—30 miles toward Port Burwell from George River: (1); Koksoak River mouth: (7); 15 miles ENE. Gyrfalcon Islands: (3); 58: 5F; 59: 14M, 29F; off Whale River: (1)

1949—103: 25M, 36F; 106: 1M, 1F; 107: 1M, 1F; Port Burwell: (6) 1M; 105: (20) 11M, 8F; Button Islands: (5); 123: (123) 3Juv, 8M, 11F; 124: 10Juv, 1M, 1F; 126: 1F; 128: (2); 103: 3M, 2F

1950—Cape Hopes Advance: (3); 206: 1F; 208: (15) 4M, 10F; 210: 6M, 11F; 216: 3F; 222: (29) 11M, 17F; 224: (18) 2M, 8F; 226: 2M, 1F; Resolution Island: 4M, 17F

Arctic, subarctic, and boreal; circumpolar. Southward to the Skaggerak and Hebrides, and to Cape Cod in North America; Behring and Okhotsk Seas and the Aleutian Islands. Depths 0 to 930 meters (Holthuis, 1947 (10)).

Spines on rostrum $\frac{0-6}{0-6}$, zero to five of which are on the carapace, with 2 to 10 pairs of spines on the telson, laterally, in these specimens; $\frac{0-8}{1-5}$, with seven to nine pairs of spines on the telson, has been given for other areas (Rathbun, 1929 (12)). In males only, the rostrum and carapace are occasionally entirely free of spines and the blade of the rostrum is reduced and thickened dorsally. This appears to be a condition present in mature animals only. It has been referred to as a variation with a reduced rostrum (Rathbun, 1929 (12)).

9. *Eualus fabricii* (Krøyer, 1841). Holthuis, 1947, p. 10
Spirontocaris fabricii. Rathbun, 1929, p. 15, Fig. 15

In all, 416 specimens were taken: 173 by dredge and beam-trawl; 44 in stramin net which touched bottom; 39 by No. 00 plankton net which touched bottom; 6 by shrimp net; and 70 in cod, 27 in bearded seal, 37 in ringed seal, and 20 in harbor seal stomachs. Depths, where taken by dredge, etc., 10 to 275 meters. Temperatures, −1.39 to 3.40° C. Salinities, 28.49 to 33.53‰.

Occurrence at stations:

1947—7: 3M, 13F; 11: 4M, 9F; 13: 1F; 18: 27M, 9F; 20 and 21: 2M, 10F; 27: 1M, 2F; 28: 1M, 3F; 33: 7M, 11F; 44: 1F; 45: 6M, 15F; 45: (28) 6M, 18F

1948—Koksoak River mouth: (37); 53: IF; 15 miles ENE. Gyrfalcon Islands: (7) 2M; 58: 5M, 1F; 59: 15F; off Whale River: (1)

1949—102: (1); Port Burwell Hr.: 1F; 103: 1Juv., 2M; 107: 13F; Mission Cove: (1); Port Burwell: (11) 3F; 105: 1M; Button Islands: (20) 6M; 123: (14) 5Juv, 4F; off Payne Bay: (4) 3F; 124: 1M, 3F; 126: 10F; 128: 17M, 8F; 103: 3F

1950—203: 5M, 2F; 206: 2F; 208: (10) 4M, 5F; 210: 1M, 11F; 222: 1F; 224: 1M, 2F; 226: 5F; 224: (2) 1F; 236: (1); Resolution Island: 3M, 4F

Arctic, subarctic, and boreal. A Pacific species extending to west Greenland (Stephensen, 1935 (15)); from the Siberian east coast and Japanese Sea through arctic Alaska and arctic Canada to west Greenland and southward to Massachusetts Bay on the east coast of the United States. Depths 4 to 200 meters (Holthuis, 1947 (10)).

Spines on rostrum and carapace $\frac{1 - 5}{1 - 5}$, of which zero to one are on the rostrum (mostly none), and two to six pairs of spines laterally on the telson in specimens examined; $\frac{2 - 6}{1 - 5}$, zero to two on the rostrum, four pairs of spines laterally on the telson has been given for other areas (Rathbun, 1929 (12)).

10. *Eualus gaimardi* (H. Milne-Edwards, 1837). Holthuis, 1947, p. 10
Spirontocaris gaimardi. Rathbun, 1929, p. 16, Fig. 16

In all, 102 specimens were taken: 34 in dredge and beam-trawl; 1 in stramin net which touched bottom; 4 in No. 6 plankton net which touched bottom; 3 in No. 0 plankton net which touched bottom; 1 in shrimp net; and 33 in cod, 3 in bearded seal, and 23 in ringed seal stomach contents. Depths, 15 to 275 meters. Temperatures, −1.39 to 3.10° C. Salinities, 28.49 to 33.53‰.

Occurrence at stations:

1947—11: 1F; 18: 1F; 20 and 21: 1F; 33: 4Juv, 3F; 45: 3M, 9F; 45: (20) 7M, 11F

1948—15 miles ENE. Gyrfalcon Islands: (1); 59: 2F

1949—Port Burwell Hr.: (2); 103: 2F; Port Burwell: (20) 2F; 105: (10) 2M, 7F

1950—Cape Hopes Advance: (2); 201C: 1M; 202: (1); 224: 1M, 1F; 234: (3) 1M, 1F; Resolution Island: (2) 1F

Arctic, subarctic, and boreal; a boreo-arctic or pan-arctic species, circumpolar (Heegard, 1941 (9)). Southward to the North Sea, Yarmouth, and Kiel; east coast of North America to Cape Cod; west coast of North America

to Sitka; shrimp taken in greatest numbers at Point Barrow, Alaska (MacGinitie, 1955 (11)); Siberia. Depths, 10 to 900 meters (Holthuis, 1947 (10)).

Spines on rostrum $\frac{6 - 11}{2 - 5}$, two to three on carapace, and two to six pairs of spines laterally on the telson in these specimens; $\frac{5 - 10}{2 - 7}$, three to five on carapace has been given for other areas (Rathbun, 1929 (12)). These specimens were entirely without a lobe or hook on the third abdominal segment, dorsally, similar in males and females. This is thought by some to be characteristic of more southerly areas (Holthuis, 1947 (10)). However, there was a blunt lobe or tubercle on a few others which might be considered to be intermediate between the typical species and its form, *E. gaimardi belcheri*; these were included in the following group assigned to *E. gaimardi belcheri*, which had a strong hook on the lobe. In view of the occurrence together of the typical species and its form, the extremes may not be said to follow a geographical incidence or cline in these areas. Also, as far southerly as St. Mary's Bay, Newfoundland, a considerable number have been taken with a well-developed hook and lobe in all males and females (H. J. Squires, unpublished).

11. *Eualus gaimardi belcheri* (Bell, 1855). Holthuis, 1947, p. 10
Spirontocaris gaimardi belcheri. Rathbun, 1929, p. 16, Fig. 17

In all, 46 specimens were taken: 23 in dredge and beam-trawl; 6 in stramin net which touched bottom; and 7 in cod, 6 in bearded seal, 3 in ringed seal, and 1 in harbor seal stomach contents. Depths, where dredged, etc., 15 to 275 meters. Temperatures, −0.46° C. Salinities, 33.53‰.

Occurrence at stations:
1947—33: 1F; 45: 7M, 4F
1948—15 miles ENE. Gyrfalcon Islands: (1); Keglo Bay: (1)
1949—102: 2F; 103: 6M, 15F; 105: 1F; 103: 4M, 2F
1950—Cape Hopes Advance: (7); 227: (1).

Arctic, subarctic, and boreal; boreo-panarctic; circumpolar (Heegard, 1941 (9)). Southward to the North Sea, and on the east coast of North America to Cape Cod, also on the west coast at Sitka; Point Barrow, Alaska (MacGinitie, 1955 (11)); Siberia. The distribution of this form follows closely that of the typical *E. gaimardi* (Heegard, 1941 (9)).

Spines on the rostrum $\frac{5 - 8}{3 - 5}$, three to four on carapace, and four to six pairs of spines laterally on the telson—not always paired—in these specimens; $\frac{8 - 12}{3 - 5}$, two to four on the carapace, has been given for other areas (Rathbun, 1929 (12)). All these specimens had a lobe or tubercle mostly produced as a hook on the third abdominal segment in males and females.

12. *Eualus macilentus* (Krøyer, 1841). Holthuis, 1947, p. 11
Spirontocaris macilenta. Rathbun, 1929, p. 16, Fig. 18

Only four specimens were taken: one by dredge and three in stomach contents of ringed seal. Depths of dredging, 55 to 73 meters.

Occurrence at stations:
1949—Port Burwell Hr.: 1F; 107: 1F; Port Burwell: (1)
1950—Cape Hopes Advance: (1)

Arctic and subarctic. A Pacific species to west Greenland (Stephensen, 1935 (15)), south to Nova Scotia. Alaska, Okhotsk Sea, Behring Sea and Strait to the Siberian Polar Sea. Depths 150 to 540 meters (Holthuis, 1947 (10)).

Spines on rostrum $\frac{14 - 16}{2 - 3}$, one to three on the carapace and three pairs of spines laterally on the telson in two females examined; $\frac{9 - 16}{1 - 4}$, zero to three spines on carapace and with three pairs of spines laterally on the telson, has been given for other areas (Rathbun, 1929 (12)).

Family CRANGONIDAE

13. *Argis dentata* Rathbun, 1902. Rathbun, 1929, p. 21, Fig. 27
Nectocrangon lar Owen, 1839 (in part). Stephensen, 1935, p. 13
Not *N. lar* (Owen), Rathbun, 1904, p. 137, Fig. 74

Specimens taken number 340; 67 by dredge and beam-trawl; and 7 in cod, 253 in bearded seal, 6 in ringed seal, and 7 in harbor seal stomach contents. Depths, where taken by dredge and beam-trawl, were 18 to 130 meters. Temperatures, where taken, were −1.22 to 2.07° C. Salinities, 29.40 to 33.42‰.

Occurrence at stations:
1947—11: 1M, 2F; 20 and 21: 3M, 1F; 33: 1M, 1F; 45: 3F; 45: (6) 5F
1948—30 miles toward Burwell from George River: (21); Koksoak River mouth: (50) 4F; 15 miles ENE. Gyrfalcon Islands: (63) 7M, 10F; off Leaf Bay: (3); 74, 77, and 78: 1M; off Whale River: (9) 1F
1949—102: 2M, 22F; 107: 1M, 2F; 105: (3) 2F; Button Islands: (7); off Payne Bay: (1); 126: 1M, 5F
1950—Cape Hopes Advance: (32) 5M, 1F; 201C: 1M; 202: (44) 4M, 8F; 203: 5Juv, 4M, 11F; 212: (2); 222: 1M; 236: (31) 4M, 4F

Arctic, subarctic, and boreal; a Pacific species (Heegard, 1941 (9)). Behring Sea southward to southeast coat of Kamchatka and Plover Bay, Siberia, and Aleutian Islands and the Alaska Peninsula to Sitka; (Canadian Arctic) and the Atlantic coast of North America from Greenland to Nova Scotia (De Man, 1920 (2)). Depths 11 to 176 meters (Rathbun, 1904 (12)).

The rostrum is reduced and there is always a line of three spines dorsally on the carapace. Dr. M. J. Rathbun's named specimens of *Argis dentata* and *A. lar*, including type specimens of *A. dentata*, at the United States

National Museum show that these two species are quite distinct. The dorsal carinae of the sixth abdominal segment of specimens of *A. lar* are rounded posteriorly, but in *A. dentata* these carinae are pointed posteriorly to form a tooth in many and in some are merely pointed but not rounded. The latter condition was present in all specimens collected by the *Calanus* Expeditions in Ungava Bay, 1947 to 1950 (Table I). It is not known whether the European authors (Hansen, 1908 (8); Stephensen, 1935 (15); Heegard, 1941 (9)), who consider *A. dentata* and *A. lar* to be synonymous, examined specimens of *A. lar* from the Pacific. Dr. Rathbun examined specimens from Greenland collected by the Princeton Expedition and called them *A. dentata* (Rathbun, 1904 (12, p. 139)). It is presumed, therefore, that *A. lar* is confined in its distribution to the Pacific, and has not been taken farther east than Point Barrow, Alaska (MacGinitie, 1955 (11)).

TABLE I

CONDITION OF POSTERIOR END OF DORSAL CARINAE ON THE SIXTH ABDOMINAL SEGMENT IN SPECIMENS OF *Argis dentata* COLLECTED IN UNGAVA BAY BY THE *Calanus* EXPEDITIONS, 1947 TO 1950

Males		Females	
Sharp-pointed, forming a tooth	Medium-pointed, not forming a tooth	Sharp-pointed, forming a tooth	Medium-pointed, not forming a tooth
14	5	22	27

14. *Sclerocrangon boreas* (Phipps, 1774). Rathbun, 1929, p. 20, Fig. 25

Specimens taken number 227; 9 by dredge, and 1 in cod, 174 in bearded seal, 39 in ringed seal, and 4 in harbor seal stomach contents. Depths, where taken by dredge, 18 to 91 meters. Temperatures, 1.90 to 2.07° C. Salinities, 29.40 to 31.87‰.

Occurrence at stations:

1947—30: 1M; 33: 2M, 1F; Button Islands: (1)
1948—Koksoak River mouth: (45) 5M, 9F; 15 miles ENE. Gyrfalcon Islands: (3) 1F; off Whale River: (38) 3F
1949—105: 1F; Button Islands: (4) 2F; off Payne Bay: 1F; 126: 1F
1950—Cape Hopes Advance: (8) 1F; 202: (32) 8M, 5F; 203: 1M, 2F; 205: (1); 222: 1F; 236: (83) 20F

Arctic, subarctic, and boreal. Widely distributed in the arctic but not known to be circumpolar; it is found also in boreal areas (Heegard, 1941 (9)). Arctic Siberia near Behring Strait and arctic Alaska (most abundant species of large shrimp taken at Point Barrow (MacGinitie, 1955 (11)); southward via Behring Sea to Kilesnov, and the Straits of Georgia, British Columbia; in the Atlantic from Cape Cod northward to the Canadian Arctic from Baffin Land to Melville Island; west and east Greenland, Iceland, the Norwegian coast north of the Arctic Circle, Spitzbergen, Bear Island, and Novaja Zelmya. Depths 0 to 400 meters (Heegard, 1941 (9)).

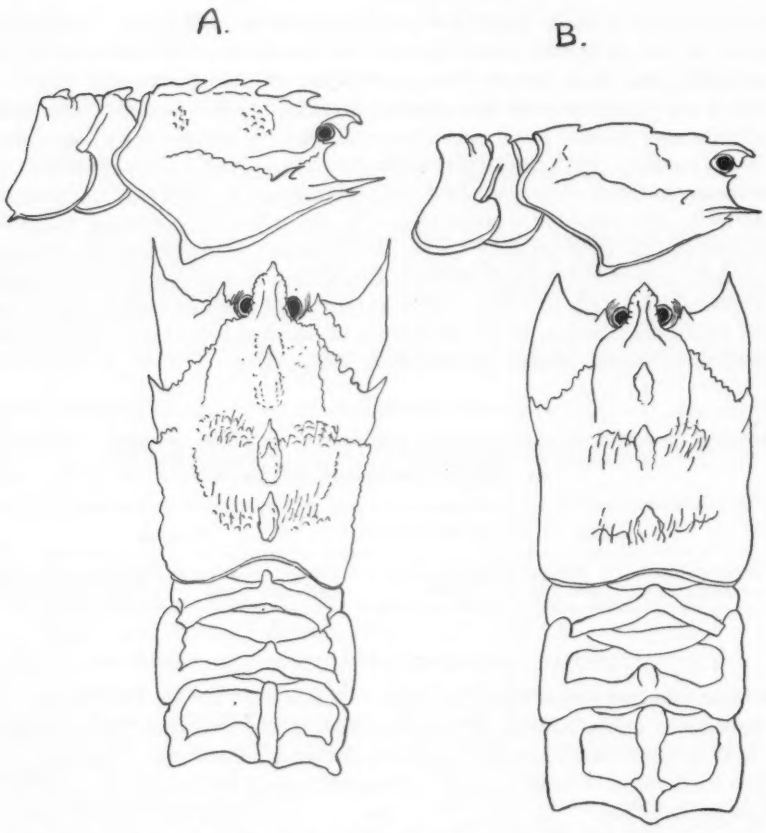

FIG. 1. Diagram of female *Sclerocrangon boreas*, 26 mm. carapace length, typical of Ungava Bay specimens (A); and of female, 25 mm. carapace length, Change Islands, Newfoundland (B).

All specimens had constant characters which differed fairly considerably from those given for the species by Rathbun (1929 (12)), and from specimens like the typical ones described by Rathbun, taken in shallow water in Newfoundland (H. J. Squires, unpublished). However, *Albatross* specimens taken off Newfoundland correspond with the Ungava Bay specimens and intergradations exist in the species material in the United States National Museum which would suggest that these forms are not separate species (Fenner A. Chace, Jr., personal communication). A table of comparison (Table II) and a figure (Fig. 1) show the main differences.

15. *Sabinea septemcarinata* (Sabine, 1824). Rathbun, 1929, p. 22, Fig. 28

Only 12 specimens were taken: 4 by dredge and beam-trawl; and 7 in cod and 1 in bearded seal stomach contents. Depths, where dredged and trawled,

TABLE II

COMPARATIVE CHARACTERS IN A TYPICAL SPECIMEN OF *Sclerocrangon boreas* FROM UNGAVA BAY, COLLECTED BY THE *Calanus* EXPEDITIONS, 1947 TO 1950, AND A SPECIMEN FROM CHANGE ISLANDS, NEWFOUNDLAND, COLLECTED IN 1948, TYPICAL OF THOSE DESCRIBED BY RATHBUN (12), 1929

Female, 26 mm. carapace length, typical of Ungava Bay specimens	Female, 25 mm. carapace length, from Change Islands, Nfld.
Carapacial spines, 3, center one double, high	Carapacial spines, 3, center one single, low
Branchiostegal spines wide-spreading to outside outline of carapace width; long, exceeding rostrum and outer spine on peduncle of antenna	Branchiostegal spines not wide-spreading, included in outline of carapace width; hardly exceeding rostrum and about equal anteriorly to outer spine on peduncle of antenna
Pterygostomian spine reaching further anteriorly than antennal spine	Pterygostomian spine about even with antennal spine
Hepatic spines not included in outline of carapace width	Hepatic spines included in outline of carapace width
Tubercle dorsally on 1st abdominal segment projecting anteriorly higher than carapace edge	Tubercle dorsally on 1st abdominal segment not produced higher than carapace edge
Pleuron of 2nd abdominal segment has a tooth directed posteriorly	No tooth on pleuron of 2nd abdominal segment
Variable number of teeth on pleura of abdominal segments in some specimens	One tooth on each pleuron of abdominal segments

18 to 130 meters. Temperatures, −1.22 to 3.10° C. Salinities, 28.78 to 33.42‰.

Occurrence at stations:
1947—33: 1F; 45: (3) 1M, 1F
1949—102: 1F; 105: (4) 1F
1950—201C: 1F; 202: 1F; 222: 1F

Arctic and subarctic; an arctic circumpolar species (Stephensen, 1935 (15)). Siberian Polar Sea to the Kara Sea, Spitzbergen and Barents Sea, White Sea, Murman Sea, west coast to Norway to the Lofotens and farther south; Iceland; east and west Greenland as far north as Discovery Bay and Grinnell Land; north of Canada (115 to 141° W. long.); south to Massachusetts Bay, east coast of North America. Not known from the Pacific. Depths, 0 to 245 meters (De Man, 1920 (2); Stephensen, 1935 (15)). Taken off Point Barrow, Alaska (MacGinitie, 1955 (11)). The collections of the *Calanus* Expeditions establish its occurrence in Ungava Bay for the first time.

FAMILY EUPAGURIDAE

16. *Eupagurus krøyeri* Stimpson, 1857. Smith, 1879, p. 48; Hansen, 1908, p. 28

Pagurus krøyeri. Rathbun, 1929, p. 27, Fig. 36

Seventy specimens were taken: 60 by dredge and beam-trawl; 1 in stramin net which touched bottom; and 9 in stomach contents of cod. Depths, 15 to 275 meters. Temperatures, −1.18 to 3.10° C. Salinities, 29.76 to 33.53‰.

Occurrence at stations:
1947—11: 1M, 2F; 28: 2F; 33: (17) 8M, 8F; 45: 1M, 2F; 45: (2) 1F
1948—59: 2M; 74, 77, and 78: 1M
1949—103: 2M, 1F; 106: 1Juv, 1M, 4F; 107: 3F; 105: (6); 126: (2) 1M; 103: 1F
1950—201C: 7M, 7F; 203: 1M; 226: 2M, 1F; 231: 1F

Arctic, subarctic, and boreal. An Atlantic species, low-arctic, boreal, more arctic than *E. pubescens* (Smith, 1879 (14)). Greenland to Stellwagen's Bank, east coast of North America, in deeper water to the south; northern Canada; northern Europe. Depths, 5 to 550 meters (Smith, 1879 (14); Rathbun, 1929 (12)).

All the specimens examined were invariably similar to the type. Hansen (1908 (8)) believed this species to be synonymous with *E. pubescens* because he stated that he had found intergrading specimens. Both species occur without intergradations as far as observed in the Newfoundland area (H. J. Squires, unpublished).

Family MAJIDAE

17. *Hyas coarctatus* Leach, 1815. Rathbun, 1929, p. 37, Fig. 51

Seventy-eight specimens were taken: 37 by dredge and beam-trawl; and 10 in cod, 1 in ringed seal, 20 in bearded seal, 4 in harbor seal, and 3 in sculpin stomachs; and 3 by hand on the shore. Depths, 0 to 130 meters. Temperatures, −1.39 to −1.22° C. Salinity, 33.42‰.

Occurrence at stations:
1947—20 and 21: (1); 28: 1M; 45: 1M, 1F; 45: 3F
1948—Leaf Bay: 1M; 74, 77, and 78: 1M; 70: 1M; Port Burwell: 3F
1949—102: 1M; 107: 1F; 105: 2M, 1F; Button Islands: (4); off Payne Bay: (4); 126: 7M, 11F
1950—Cape Hopes Advance: (2); 202: (7); 204: (1); 206: 1M; 208: 2M, 1F; 210: 2M, 2F; 212: (3); 215: (1); 216: (2) 1F; 224: 1F; 226: 2F; 224: (2) 1F

Arctic, subarctic, and boreal. The typical form is boreo-lower arctic (Heegard, 1941 (9)). Atlantic: west Greenland, Hudson Bay, east coast of North America to North Carolina; Iceland up to 66½° N. lat.; northern Europe to 79½° N. lat., southward to the English Channel. Arctic: Mackenzie River, Alaska, Siberian coast to Bennett Island. Pacific: Behring Sea to west coast of Alaska and to Korea. Depths, 3 to 900 fathoms (Heegard, 1941 (9)). *H. coarctatus alutaceus* has been recorded in the Pacific, Arctic, and West Atlantic (Rathbun, 1929 (12)), but this form was not present in the *Calanus* Expeditions collections.

The ratio of carapace length to rostrum length has been used to separate the typical *H. coarctatus* from the form *H. coarctatus alutaceus*. In *H. coarctatus alutaceus* the carapace length is 7.1 to 9.3 times as long as the rostrum length,

TABLE III

RATIO OF CARAPACE LENGTH TO ROSTRUM LENGTH IN *Hyas coarctatus* COLLECTED IN UNGAVA BAY BY THE *Calanus* EXPEDITIONS, 1947 TO 1950

Av. carapace length, mm.	Carapace × rostrum	No. specimens examined
7	2.3	2
12	4.0	1
17	3.4	2
22	4.4	1
27	4.5	8
32	4.6	4
37	5.3	6
42	5.3	7
47	6.0	3
52	6.5	1
57	—	—
62	6.2	1
67	—	—
72	—	—
77	—	—
82	—	—
87	7.3	1

while this ratio is 4.5 to 6.4 times in the typical *H. coarctatus*, carapace lengths 30 to 52 mm. (Rathbun, 1929 (12)). The latter ratios are similar to those found for specimens collected in the Ungava Bay area (Table III).

Lengths and Maturities of Species

Lengths

Each specimen was measured when possible in order to give sizes of the animals which would allow comparisons with those of specimens from other areas (a basis of comparison is the length of each species when first mature; Chace, 1940 (1)). A regional comparison is not attempted in this paper. Since whole lengths, however, are given by many authors, while carapace lengths are used in this paper, regression equations are given for conversion of carapace lengths to whole lengths. These equations were calculated from sight curves drawn to points plotted from average whole lengths at each carapace length (Figs. 2 to 4). The curves in Figs. 2 to 4 were weighted according to the number of measurements supporting each average; measurements of males and females were combined for these curves.

It is striking that in the shrimp species collected, all, with the possible exception of *Pasiphaea tarda*, had females which exceeded the males in length (Figs. 2 to 4). This was particularly evident in species taken in large quantity such as *Lebbeus polaris*, *Eualus fabricii* (Fig. 3), and *Lebbeus groenlandicus* (Fig. 2). It was less evident in *Eualus gaimardi belcheri* (Fig. 4). In hermit crabs (*Eupagurus krøyeri*) and spider crabs (*Hyas coarctatus*) the males generally exceeded the females in length (Fig. 4), although average lengths were not greatly different. The array of lengths in female shrimp, also, shows considerably greater scatter than in males and possibly a greater growth rate in females. The generally smaller size of males compared with females in

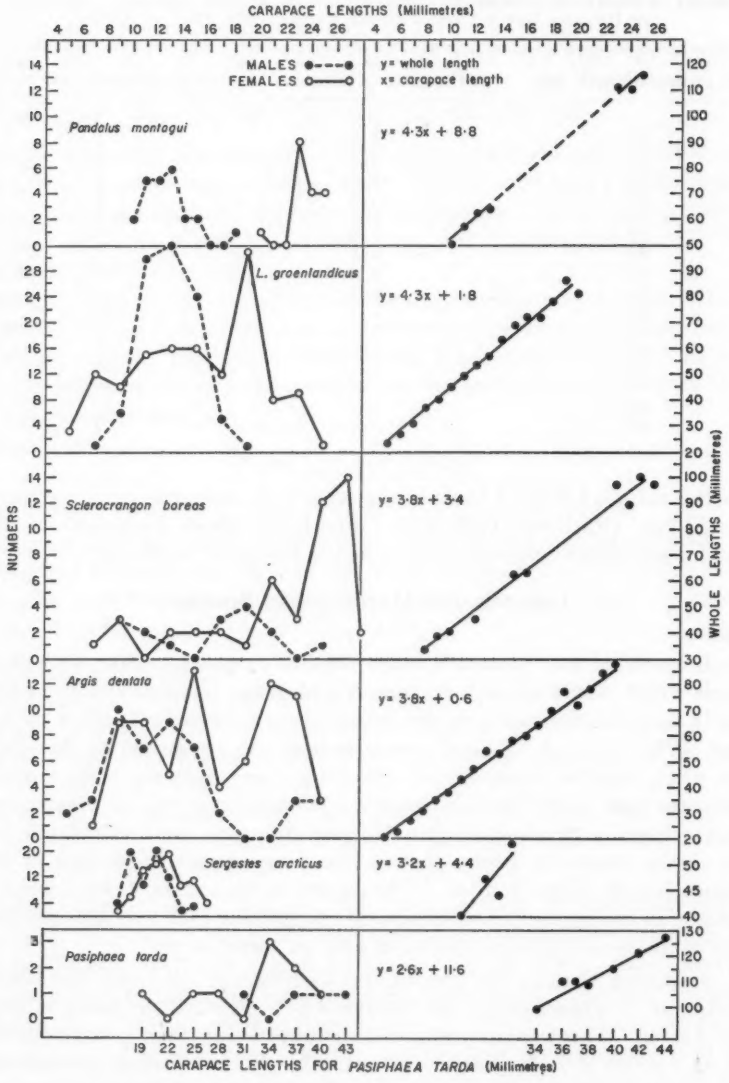

FIG. 2. Carapace length frequencies and regression curves for conversion of carapace lengths to whole lengths in *Pandalus montagui*, *Lebbeus groenlandicus*, *Sclerocrangon boreas*, *Argis dentata*, *Sergestes arcticus*, and *Pasiphaea tarda*. *Calanus* Expeditions in Ungava Bay, 1947 to 1950.

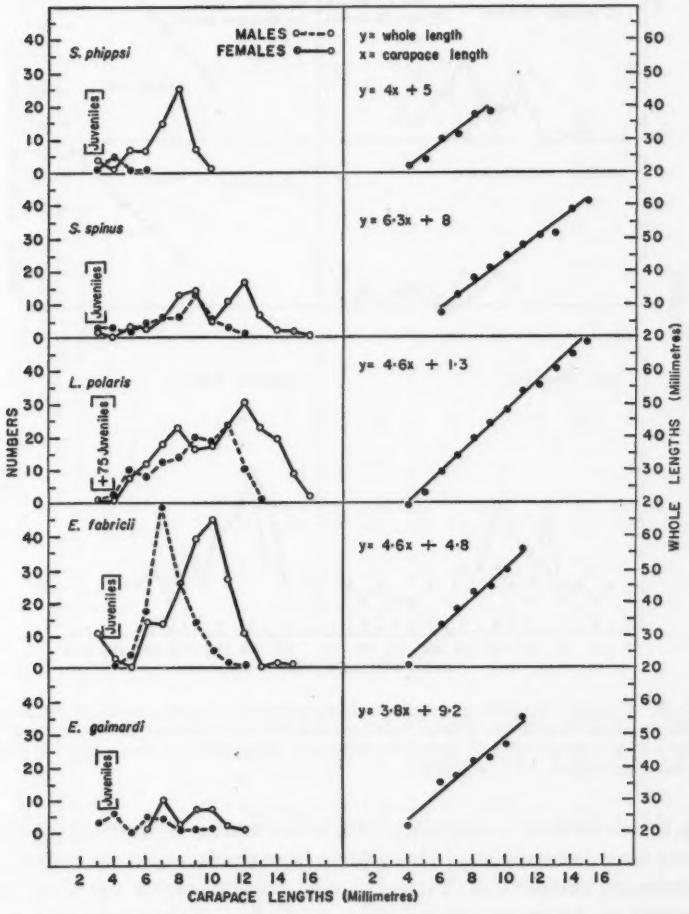

FIG. 3. Carapace length frequencies and regression curves for conversion of carapace lengths to whole lengths in *Spirontocaris phippsi*, *Spirontocaris spinus*, *Lebbeus polaris*, *Eualus fabricii*, and *Eualus gaimardi*. *Calanus* Expeditions in Ungava Bay, 1947 to 1950.

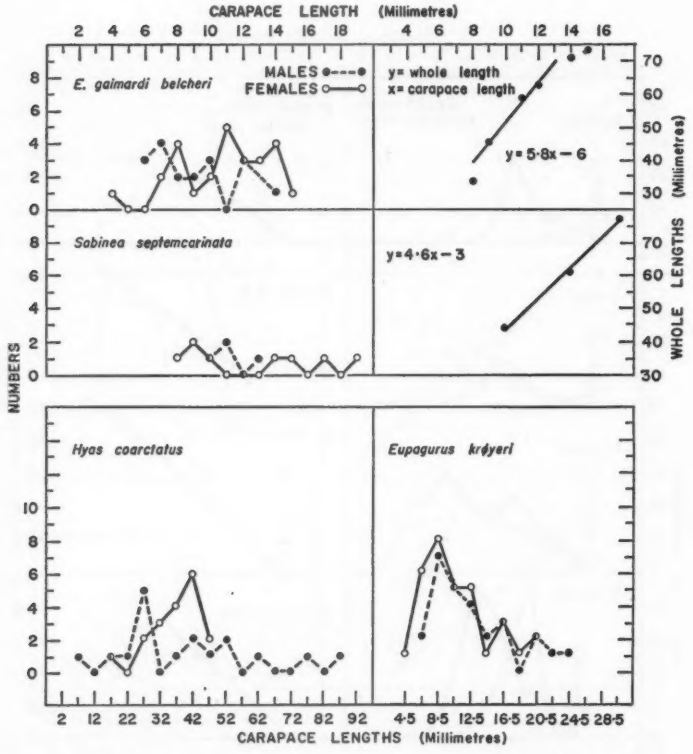

FIG. 4. Carapace length frequencies and regression curves for conversion of carapace lengths to whole lengths in *Eualus gaimardi belcheri* and *Sabinea septemcarinata*; and carapace length frequencies of *Hyas coarctatus* and *Eupagurus krøyeri*. *Calanus* Expeditions in Ungava Bay, 1947 to 1950.

shrimp seems to show a similarity with other marine animals of which some fishes are notable examples. This follows from the fact that the males mature at a somewhat smaller size (Table IV), and possibly earlier age than females, and growth is slower as a consequence thereafter (Templeman and Squires, 1956 (17)).

The shrimp, *Pandalus montagui*, presents a special case where the animals are protandrous (Thorson, 1946 (18, p. 325)). No males taken exceeded 18 mm. in carapace length, and females taken were not less than 20 mm. in carapace length, and reached an extreme length of 25 mm. (Fig. 2).

The largest specimens of shrimp taken belonged to the species *Pasiphaea tarda*, with carapace lengths of 27 to 42 mm. The smallest and, incidentally, most numerous specimens were in the family Hippolytidae, of which *Spirontocaris phippsi* (3 to 10 mm. carapace length) and *S. spinus* (3 to 15

TABLE IV

CARAPACE LENGTHS AND AVERAGE EGG DIAMETER (MM.) IN OVARY OF SHRIMP COLLECTED BY THE *Calanus* EXPEDITIONS IN UNGAVA BAY, 1947 TO 1950, AND INDICATION OF FIRST MATURITY (EGG DIAMETER IN ITALICS INDICATES LENGTH AT WHICH FEMALES OF EACH SPECIES WERE OBSERVED TO BE FIRST MATURE. AN ASTERISK INDICATES THE LENGTH AT WHICH MALES OF EACH SPECIES WERE OBSERVED TO BE FIRST MATURE; NUMBER IN PARENTHESES = NUMBER OF INDIVIDUALS FROM WHICH EGGS WERE MEASURED)

Species	Carapace lengths, mm.																							
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
Pandalus montagui	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0 (2)	—	—	—
Spirontocaris phippsi	—*	0.3 (2)	*0.4* (3)	0.5 (7)	0.5 (5)	0.5 (1)	1.1 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Spirontocaris spinus	—	—	0.3* (2)	0.3 (3)	*0.5* (4)	0.3 (11)	0.5 (4)	0.6 (8)	0.7 (15)	0.8 (6)	0.3 (1)	0.5 (2)	—	—	—	—	—	—	—	—	—	—	—
Lebbeus groenlandicus	—	—	—	—	0.3 (4)	0.4 (3)	0.5* (5)	0.4 (5)	0.4 (4)	0.5 (6)	*0.7* (6)	1.1 (4)	1.4 (5)	1.2 (4)	0.9 (14)	1.0 (6)	1.1 (3)	0.8 (1)	2.0 (3)	1.3 (1)	—	—	—
Lebbeus polaris	—	—	0.6 (4)	0.4 (12)	0.4* (14)	0.3 (6)	*0.7* (12)	0.7 (13)	1.0 (25)	0.9 (20)	1.0 (17)	0.9 (10)	0.4 (2)	—	—	—	—	—	—	—	—	—	—
Eualus fabricii	—	—	—*	0.3 (5)	*0.4* (6)	0.5 (8)	0.7 (18)	0.6 (10)	0.4 (2)	0.3 (1)	0.5 (1)	—	—	—	—	—	—	—	—	—	—	—	—
Eualus gaimardi	—	—	0.3 (1)	0.3* (4)	0.3 (2)	0.3 (3)	*0.3* (4)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Eualus gaimardi belcheri	—	—	—	—	0.3 (2)	—*	0.3 (1)	0.3 (5)	*1.3* (1)	0.7 (2)	1.2 (4)	0.5 (1)	—	—	—	—	—	—	—	—	—	—	—
Eualus macilentus	—	—	—	—	—	—	—	0.8 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Argis dentata	—	—	—	—	0.2 (2)	0.4* (3)	0.4 (2)	0.3 (1)	0.4 (1)	0.3 (6)	0.2 (4)	—	*0.4* (4)	1.1 (2)	1.0 (2)	1.6 (5)	—	1.7 (2)	0.6 (1)	—	—	—	—
Sclerocrangon boreas	—	—	—	—	—	0.3* (1)	—	—	0.4 (1)	—	—	—	—	—	—	—	—	—	—	1.0 (1)	—	—	1.5 (1)
Sabinea septemcarinata	—	—	—	—	—	—	0.3* (1)	—	—	—	*1.3* (1)	—	—	—	—	—	—	—	—	—	—	—	—

mm. carapace length) were the smallest. *Lebbeus groenlandicus* (5 to 25 mm. carapace length) was the largest hippolytid. In the specimens examined *Eualus gaimardi* was somewhat smaller (3 to 12 mm. carapace length) than its closely related form *E. gaimardi belcheri* (6 to 15 mm. carapace length). Specimens belonging to the family Crangonidae compare in size with the largest hippolytid; its most abundant species, *Argis dentata*, which ranges in carapace length from 4 to 25 mm. and *Sclerocrangon boreas* (6 to 29 mm. carapace length) were among the largest specimens of shrimp taken in the shallow areas of Ungava Bay.

Maturities

Gross approximation of maturity was made from diameter of eggs in ovary or on pleopods, and size of appendices masculinae compared with appendices internae (Chace, 1940 (1)). Some shrimp which had eggs on the pleopods in June also had large eggs in the ovary which would be extruded, undoubtedly, later in the season; however, some which were ovigerous in July and had but small eggs in the ovary were presumed to have just spawned, and would carry eggs through the following winter. Most species had spawned by July, although some specimens collected in September still had large eggs in the ovary (Table V). Generally, smaller or first maturing females spawned earlier in the year than larger females that were presumably already ovigerous in early summer. There was evidence that mature females spawned each year: some with the ovary full of large eggs also had eggs on the pleopods which evidently had not yet hatched. Most early stage larvae were taken in June and July only.

The largest eggs in these species were carried by *Sclerocrangon boreas* (3.1 mm., average diameter in eggs from seven specimens) and *Pasiphaea tarda* (2.7 mm., average diameter in eggs from six specimens). Among shrimp the smallest eggs were carried by *Eualus* and *Spirontocaris* (about 1.2 mm. average egg diameter in 47 specimens), which were the smallest species present in general. *Lebbeus groenlandicus* and *L. polaris*, also in the family Hippolytidae, had larger eggs than *Eualus* or *Spirontocaris* (about 2.2 mm. average egg diameter in 48 specimens). The crangonids *Argis* and *Sabinea* both had eggs about 2.0 mm. in average diameter in four specimens examined. The hermit crabs (*Eupagurus*) and spider crabs (*Hyas*) had eggs which averaged less than 1.0 mm. in diameter in 10 and 15 specimens, respectively (Table VI).

Large shrimp in any species appeared to have larger eggs than small shrimp (Table VI) but it was observed generally that eggs were, presumably, somewhat less in diameter when first extruded than later, and were largest previous to hatching, namely, when there were large eggs in the ovary as well as on the pleopods (Table V). A considerable number of larger shrimp taken in the earlier months carried eggs spawned the previous year: these eggs were large. Large shrimp taken later in the year sometimes carried slightly smaller eggs.

TABLE V

AVERAGE EGG DIAMETERS (MM.) IN OVARY AND ON PLEOPODS OF SHRIMP AND CRABS COLLECTED BY THE *Calanus* EXPEDITIONS IN UNGAVA BAY, JUNE TO SEPTEMBER, 1947 TO 1950 (NUMBER IN PARENTHESES = NUMBER OF INDIVIDUALS FROM WHICH EGGS WERE MEASURED)

Species	June		July		August		September		Av. egg diameter on pleopods
	In ovary	On pleopods	In ovary	On pleopods	In ovary	On pleopods	In ovary	On pleopods	
Pasiphaea tarda	—	—	—	—	0.2 (1)	2.6 (5)	2.5 (1)	3.0 (1)	2.7
Pandalus montagui	—	—	1.0 (2)	—	—	—	—	—	—
Spirontocaris phippsi	—	—	0.4 (11)	1.4 (5)	0.6 (8)	1.2 (5)	—	1.4 (2)	1.3
Spirontocaris spinus	0.4 (1)	1.4 (1)	0.5 (32)	1.4 (11)	0.6 (23)	1.5 (5)	—	—	1.4
Lebbeus groenlandicus	0.3 (2)	—	0.9 (49)	2.3 (7)	0.6 (20)	2.0 (1)	1.8 (3)	—	2.2
Lebbeus polaris	0.5 (3)	2.0 (1)	0.8 (96)	2.2 (24)	0.8 (21)	2.0 (10)	0.5 (15)	2.0 (5)	2.1
Eualus fabricii	0.9 (1)	—	0.6 (30)	1.2 (2)	0.4 (16)	1.2 (8)	0.4 (4)	0.9 (3)	1.1
Eualus gaimardi	—	—	0.3 (3)	1.0 (1)	0.3 (10)	1.2 (4)	0.3 (1)	—	1.2
Eualus gaimardi belcheri	—	—	0.7 (15)	—	0.3 (1)	1.3 (1)	—	—	1.3
Eualus macilentus	—	1.2 (1)	0.8 (1)	—	—	—	—	—	1.2
Argis dentata	—	—	0.7 (31)	2.0 (3)	0.7 (7)	—	—	—	2.0
Sclerocrangon boreas	—	—	0.4 (2)	3.0 (1)	1.3 (2)	3.3 (2)	—	3.1 (6)	3.1
Sabinea septemcarinata	—	—	0.3 (1)	—	1.3 (1)	2.0 (1)	—	—	2.0
Eupagurus krøyeri	—	0.9 (2)	—	0.9 (5)	—	0.9 (3)	—	—	0.9
Hyas coarctatus	—	—	—	0.7 (4)	0.8 (1)	0.7 (11)	—	—	0.7

TABLE VI

AVERAGE EGG DIAMETER (MM.) ON PLEOPODS OF SHRIMP AT EACH CARAPACE LENGTH COLLECTED BY THE *Calanus* EXPEDITIONS IN UNGAVA BAY, 1947 TO 1950 (NUMBER IN PARENTHESES IS NUMBER OF INDIVIDUALS FROM WHICH EGGS WERE MEASURED)

Species	Carapace lengths, mm.																					Range of egg diameters, mm.	Av. egg diameter, mm.
	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	24	25	26	27	28		
Spirontocaris phippsi	1.3 (1)	1.3 (3)	1.4 (7)	—	1.5 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.1–1.5	1.3
Spirontocaris spinus	—	—	1.2 (2)	1.4 (2)	1.4 (3)	1.5 (1)	1.5 (3)	1.6 (3)	1.6 (1)	1.6 (1)	1.4 (1)	—	—	—	—	—	—	—	—	—	—	1.0–1.8	1.4
Lebbeus groenlandicus	—	—	—	—	—	—	—	—	—	—	—	—	2.2 (5)	2.3 (2)	2.4 (1)	—	—	—	—	—	—	2.0–2.4	2.2
Lebbeus polaris	—	—	—	—	—	2.0 (5)	2.1 (10)	2.2 (11)	2.1 (8)	2.1 (4)	2.3 (2)	—	—	—	—	—	—	—	—	—	—	1.8–2.4	2.1
Eualus fabricii	—	—	—	1.2 (3)	1.1 (6)	1.2 (1)	0.9 (2)	1.2 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—	0.9–1.2	1.1
Eualus gaimardi	—	—	—	—	1.2 (3)	1.1 (2)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.0–1.2	1.2
Eualus gaimardi belcheri	—	—	—	—	—	—	1.3 (1)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.3	1.3
Argis dentata	—	—	—	—	—	—	—	—	—	—	—	—	—	2.0 (1)	—	2.0 (2)	—	—	—	—	—	2.0	2.0
Sclerocrangon boreas	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3.0 (2)	3.0 (2)	3.3 (2)	3.1 (2)	3.5 (1)	2.9–3.5	3.1

Assessment of the Decapod Fauna of Ungava Bay as Shown by the *Calanus* Expeditions Collections

The percentage of dredgings, etc., which took decapods was very high (average 95%) in the 1947 to 1950 *Calanus* Expeditions in Ungava Bay. The percentage of stations at which decapod larvae were taken in plankton hauls was also high (average 85%, Fig. 5). The presence of many specimens in stomach contents of fish and seals also attests the considerable quantity of decapods and their importance in the bionomics of the area.

In comparison with adjacent areas, Ungava Bay has a decapod fauna similar to that of the shallow water areas of west Greenland and has a few subarctic–boreal elements not found in the Hudson Bay area, as far as is known from collections in these areas. Ungava Bay also shows some incursion from the east of deep water pelagic species, notably, *Pasiphaea tarda* and *Sergestes arcticus*. These species were taken near Port Burwell and Resolution Island and evidently do not range far into Hudson Strait. Other deep-water pelagic species of Davis Strait and west Greenland have not been taken in the present collections. Although current movements are predominantly out of the bay and southward along the Labrador coast (Dunbar, 1951 (3)), there is evidence of Atlantic water in Ungava Bay from hydrographic and plankton results already discussed in this series (Fontaine, 1955 (6)). The presence of *Pasiphaea* and *Sergestes* in the *Calanus* Expeditions collections in this area, 1947 to 1950, supports such a conclusion.

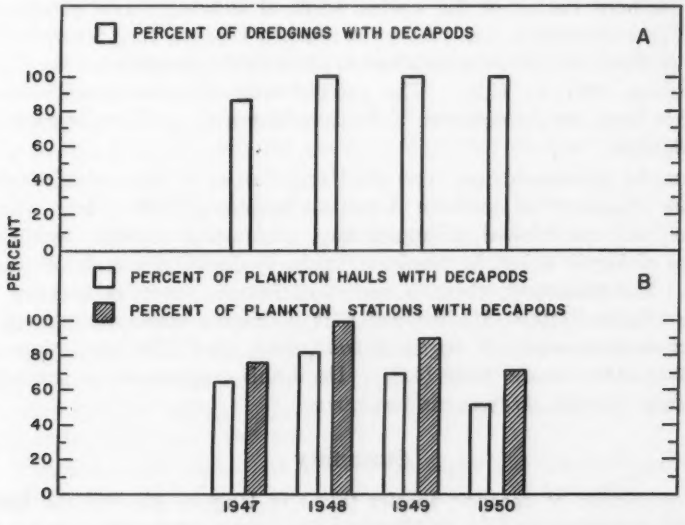

FIG. 5. Percentage of dredgings (A), and percentage of plankton hauls and plankton stations (B), in which decapods were taken in Ungava Bay by the *Calanus* Expeditions, 1947 to 1950.

The more typical decapod fauna of Ungava Bay is represented by such species as *Argis dentata*, *Lebbeus groenlandicus*, *L. polaris*, *Eualus fabricii*, *Spirontocaris spinus*, *Sclerocrangon boreas*, *Eupagurus krøyeri*, and *Hyas coarctatus*. These were taken in considerable numbers in dredgings, etc., and as a major constituent of the stomach contents of fish and seals in Ungava Bay, and were well-distributed throughout the area explored. Larvae, also, of these species (except *Sclerocrangon*, which does not have pelagic larvae) were taken in considerable numbers in plankton hauls. No larvae of *Sergestes* or *Pasiphaea* were taken in the Bay or area explored near its entrance during 1947 to 1950.

Two dominant forms in the area, *Argis dentata* and *Lebbeus groenlandicus*, have originated in the Pacific (Stephensen, 1935 (15)), and it is easy to see that Ungava Bay can be more readily populated by the spreading of species from the west because of water movements (Dunbar, 1951 (3)). Other Pacific species, *Eualus fabricii* and *E. macilentus*, are represented in these collections, although *Chionoecetes opilio* is not: the latter may be restricted to deeper water than was explored by the *Calanus* Expeditions in Ungava Bay. Decapods defined by Stephensen (1935 (15)) as exclusively high arctic are deep water species and presumably would not be found in the shallow water areas of Ungava Bay; this would apply to other deep water but subarctic and boreal species found in nearby waters to the east. Circumpolar species, *Lebbeus polaris*, *Spirontocaris phippsi*, *S. spinus*, and *Sclerocrangon boreas* are well-represented in these collections. Subarctic–boreal species of the North Atlantic, *Hyas coarctatus*, *S. lilljeborgi*, and *Pandalus montagui*, are also found in the northern Pacific or the waters north of Behring Strait (Stephensen, 1935 (15)) so that they, too, may have reached Ungava Bay from the west. Larvae of *Hyas* were in large numbers in plankton hauls taken by the *Calanus* Expeditions, 1947 to 1950. The pelagic Atlantic species *Sergestes* and *Pasiphaea* taken at the entrance to Ungava Bay may possibly be considered as accidentals.

A decapod, presumably not from the Pacific but an Atlantic species extending from America to northern Europe (Rathbun, 1929 (12)), which is extremely well–established in Ungava Bay, as shown particularly by the large numbers of larvae taken in plankton hauls, is the hermit crab, *Eupagurus krøyeri*. Unfortunately, there is some controversy over its identity as a species. Hansen (1908 (8)) believed that *E. krøyeri* was synonymous with *E. pubescens* and perhaps *E. trigonocheirus* (which is a Pacific form taken north of Alaska; MacGinitie, 1955 (11)). Until this controversy is resolved no conclusions about *E. krøyeri* can be drawn.

Summary

1. Comparison of decapod species taken in Ungava Bay by the *Calanus* Expeditions with those taken in the west Greenland – Davis Strait area shows that there is close similarity particularly between the decapod fauna of Ungava Bay and that of the shallow water areas of west Greenland.

2. A compiled key for all species collected and their closely related forms emphasizes numbers of exopods, epipods, etc., since these characters help to identify damaged specimens from stomach contents of fish and seals.

3. Systematics of the 17 species collected is treated under occurrence in Ungava Bay, world distribution, and taxonomy—the latter referring mostly to variable characters such as number of spines on rostrum, carapace, and telson.

4. *Pasiphaea tarda* and *Sergestes arcticus*, deep-water pelagic forms of the Davis Strait – west Greenland area, were taken for the first time near the entrance to Ungava Bay.

5. *Eualus gaimardi* and its closely allied form *E. g. belcheri* were found in similar areas in Ungava Bay. A northern trend towards the form *belcheri* is shown not to apply in consideration of specimens from this collection and others in the northwestern Atlantic.

6. *Argis dentata* as defined by Rathbun was found to be the sole species of this genus in Ungava Bay, and since Dr. Rathbun named specimens from Greenland *Argis dentata*, the distribution of *A. lar* east of Alaska is questioned.

7. A constant character of a double spine in the center on the carapace was present in *Sclerocrangon boreas* and generally the spines were larger than on the typical form. However, in view of intergradations and similar specimens from areas widely separated, no new species naming was proposed.

8. The *Calanus* Expeditions, 1947 to 1950, took *Eualus macilentus* and *Sabinea septemcarinata* in Ungava Bay for the first time that has been recorded.

9. Ratio of carapace length to rostrum length showed that the specimens of *Hyas coarctatus* examined were the typical form of the species and not the form *alutaceus*.

10. Equations were calculated to convert carapace lengths to whole lengths in most species of shrimp represented.

11. Lengths of females exceeded those of males in range in all species of shrimp except *P. tarda*, and the converse was seen to apply in hermit and spider crabs.

12. *P. tarda* was the largest species of shrimp taken by the *Calanus* Expeditions, 1947 to 1950; but *L. groenlandicus*, *A. dentata*, and *S. boreas* were all of fair size and abundance in the shallow areas of the bay.

13. Male shrimp were found to mature at a much smaller size than did first mature females, which probably accounted for their smaller size in general.

14. Spawning seemed to take place earlier in the year in first maturing females—some ovigerous in June and July—than in larger mature females, whose eggs hatched mostly in June and July, and which did not spawn until August or September, for the most part.

15. Egg size varied somewhat in a species; eggs just spawned appeared to be smaller, generally (all were measured from about 7% formalin).

16. Largest eggs were carried by *Sclerocrangon* (3.1 mm. average diameter) and *Pasiphaea* (average 2.7 mm.); *Lebbeus* had eggs 2.0 to 2.5 mm., *Spirontocaris* 1.2 to 1.4 mm., *Eualus* 1.0 to 1.2 mm., *Argis* and *Sabinea* about 2.0 mm.

in average diameter; *Eupagurus* and *Hyas* had eggs less than 1.0 mm. in diameter.

17. The percentage of stations where decapods were taken in dredgings and plankton hauls was very high. Also, the occurrence of decapods in stomach contents of many fish and seals show that they occupy an important place in the fauna of Ungava Bay.

18. Some comparison of the decapod fauna of Ungava Bay with that of adjacent areas is made. The species which typify most the decapod fauna of Ungava Bay are *Argis dentata*, *Lebbeus groenlandicus*, *L. polaris*, *Eualus fabricii*, *Spirontocaris spinus*, *Sclerocrangon boreas*, *Eupagurus krøyeri*, and *Hyas coarctatus*. *L. polaris* was the most abundant of all decapod species, as in the west Greenland fauna.

19. The dominating role of Pacific species of decapods found in Ungava Bay suggests that the area was colonized by decapods from the west for the most part; the presence of Atlantic deep-water forms such as *Pasiphaea* and *Sergestes* is accidental.

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A special word of thanks is given to Dr. Fenner A. Chace, Jr., Curator of Marine Invertebrates, United States National Museum, who helped with gifts of reprints of his own work, and made available named specimens and type species of decapods for comparison at the Museum. He pointed out the wide variation in *Sclerocrangon boreas* from many areas.

Assistance in compilation of tables and in calculations were given by Miss C. H. Starks and Mr. G. E. Tucker, who also made finished drawings of the graphs. Mr. H. R. Mullett and Mr. E. L. Rowe also helped with drawings and photographs. Drs. W. Templeman and M. J. Dunbar read the manuscript. To all these people I extend my sincere gratitude.

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NOTE: Table VII (Appendix) follows.

APPENDIX

TABLE VII

NUMBERS OF SPECIMENS OF EACH SPECIES OF DECAPOD CRUSTACEANS TAKEN BY DIFFERENT MEANS IN UNGAVA BAY BY THE *Calanus* EXPEDITIONS, 1947 TO 1950

Species	Dredge and beam-trawl	Stramin (touched bottom)	No. 6	No. 0	No. 00 No. 5	Shrimp net	Stomach contents of: Cod	Ringed seal	Bearded seal	Harbor seal	Harp seal	Sculpin	By hand	Total
Sergestes arcticus	—	—	—	—	—	88	174	1	—	—	4	—	—	267
Pasiphaea tarda	—	—	—	—	—	—	46	—	—	—	—	—	—	46
Pandalus montagui	11	3	—	—	—	—	37	29	1	—	—	—	—	81
Spirontocaris lilljeborgi	—	—	—	—	—	—	—	—	2	—	—	—	—	2
Spirontocaris phippsi	24	21	—	—	—	—	18	32	36	6	—	—	—	137
Spirontocaris spinus	77	34	—	—	—	—	47	3	21	3	—	—	—	185
Lebbeus groenlandicus	159	7	—	—	—	2	40	14	176	15	—	—	—	413
Lebbeus polaris	211	23	—	—	125	19	69	13	8	5	—	—	—	473
Eualus fabricii	173	44	—	—	39	6	70	37	27	20	—	—	—	416
Eualus gaimardi	34	1	4	3	—	1	33	23	3	—	—	—	—	102
Eualus gaimardi belcheri	23	6	—	—	—	—	7	3	6	1	—	—	—	46
Eualus macilentus	1	—	—	—	—	—	—	3	—	—	—	—	—	4
Argis dentata	67	—	—	—	—	—	7	6	253	7	—	—	—	340
Sclerocrangon boreas	9	—	—	—	—	—	1	39	174	4	—	—	—	227
Sabinea septemcarinata	4	—	—	—	—	—	7	—	1	—	—	—	—	12
Eupagurus krøyeri	60	1	—	—	—	—	9	—	—	—	—	—	—	70
Hyas coarctatus	37	—	—	—	—	—	10	1	20	4	—	3	3	78
Hippolytid	1	—	—	—	—	—	2	16	27	78	—	—	—	124
Decapod	—	—	—	—	—	—	—	33	18*	—	1	—	—	52
Totals	891	140	4	3	164	116	577	253	773	143	5	3	3	3075

*Many in addition were in smaller fragments and could not be counted.

NOTES

A PRELIMINARY NOTE ON THE NEMATODE PARASITES OF SEALS IN THE GULF OF THE ST. LAWRENCE[1]

P. L. J. Montreuil and K. Ronald[2]

The incidence of nematodes (Anisakinae) has been recorded for several years from seals of the Canadian Atlantic coast. The incidence data are, in most cases, based upon stomach nematode counts. The authors have found, in a preliminary investigation, that the intestinal tract is also a site of infection for these nematodes, in numbers sometimes equal to or even exceeding those found in the stomach. These parasites are usually firmly attached to the intestinal mucosa; therefore they cannot be considered as transients. In the material already studied, there is some indication that embryonic development occurs in the small intestine. This suggests that the intestinal phase of the nematode may be essential to the normal development of the egg and embryo.

Three species of seals (gray, *Halichoerus grypus;* harp, *Phoca groenlandica;* harbor, *P. vitulina*) are noted here as carrying nematodes in their intestines. These findings indicate the need for reassessment of the available data on incidence in seals, the harp seal playing a more important role in the life cycle of some nematode fish parasites than was previously realized. This is especially so as adult harp seals captured on the ice carry nematodes in the intestine even in the absence of stomach infections.

Work is in progress towards an analysis of data and material already held. The collections to be made this year will also be used for a study of the embryonic development of these nematodes, up to the point of their expulsion from the host. The results of these studies will be published at a later date.

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